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THE AMERICAN REVIEW OF Respiratory Diseases

OFFICIAL JOURNAL OF THE AMERICAN THORACIC SOCIETY
Medical Section of the National Tuberculosis Association

1961 ANNUAL MEETING

CINCINNATI, OHIO

MAY 21–25

The 1961 Annual Meeting of the National Tuberculosis Association, the American Thoracic Society, and the National Conference of Tuberculosis Workers will be held in Cincinnati, Ohio, May 21–25.

Registration and the exhibits program will be in the Netherland Hilton Hotel. Program sessions will be held in the Netherland Hilton and cooperating hotels, each within a block of Fountain Square in the heart of the city.

The registration desk will be open beginning at 10:00 a.m., Sunday, May 21. There is no registration fee. A registration badge must be worn to ensure admission to program sessions.

Exhibits will open at 8:30 a.m., Monday, May 22, and will be on view, during morning and afternoon hours, throughout the meeting. The high caliber of exhibits, as at previous meetings, will make this a phase of the program to which all attending will want to devote a generous portion of their time.

Preceding the general meetings—ATS Committee and Council meetings will be held Saturday and Sunday, May 20 and 21. The annual Tuberculosis Nursing Advisory Service Seminar will be held on Saturday, May 20. There will be special interest sessions on Sunday, May 21.

Medical, public health, and nursing sessions will begin on Monday, May 22. General sessions will include an opening program Monday at 9:00 a.m. A keynote, awards, and NTA business session will be held Monday evening, and a closing general session at 11:00 a.m., Thursday, May 25.

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MAY 1961
VOLUME 83/NUMBER 5

THE AMERICAN REVIEW OF Respiratory Diseases

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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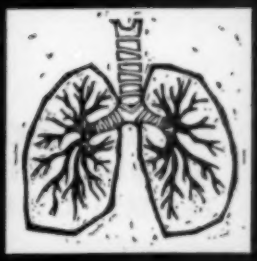

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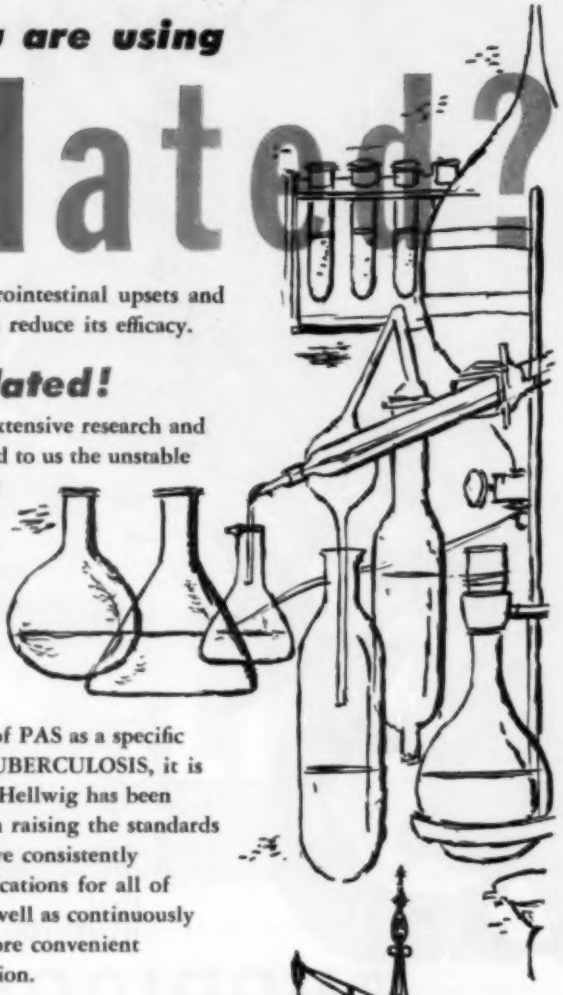

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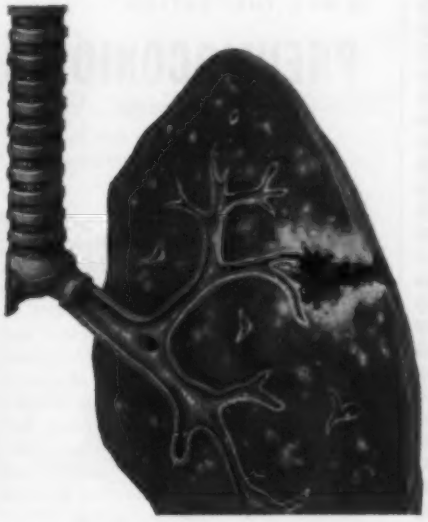

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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VOLUME 83 *May 1961* NUMBER 5

DEGENERATION OF THE CILIATED CELLS OF THE BRONCHIAL EPITHELIUM (CILIOCYTOPHTHORIA) IN ITS RELATION TO PULMONARY DISEASE[1, 2]

GEORGE N. PAPANICOLAOU, EMMA LOU BRIDGES, AND CONSTANTINE RAILEY

(Received for publication December 16, 1960)

INTRODUCTION

The term "ciliocytophthoria" (CCP) was suggested in 1956 (1) by one of the writers (G.N.P.), in a preliminary note, for designating the phenomenon of a mass degeneration of the ciliated components of the bronchial epithelium occurring in certain cases of acute and chronic pulmonary disease.

The conclusions drawn from the observations upon which this preliminary report was based were summarized as follows: (*1*) A distinct cytologic pattern reflecting an extensive degeneration and mass destruction of ciliated cells of the bronchial epithelium can be recognized in smears prepared from sputum specimens in cases of certain acute and chronic inflammatory conditions of the lung. (*2*) The cytologic examination of smears may prove valuable for the diagnosis of pulmonary infections belonging to this special group as well as for a study of their etiology and the relative value of various modes of therapy. In a relatively high percentage of ciliocytophthoria-positive cases (12 of 100), the presence of a bronchogenic carcinoma was established by clinical and pathologic findings.

Since the number of observations upon which these conclusions were based was relatively small, it was believed that the various morphologic and clinical aspects of this interesting phenomenon and their possible relationship to cancer of the lung or to pulmonary infections, particularly those of viral etiology, should be the subject of a more exhaustive investigative study. This led to the formation of a special project which was divided into three parts, each based on a different investigative approach.

One was an attempt to establish normal standards through the study of the ciliocytophthoria status in a control group consisting of men and women free from symptoms suggestive of cancer of the lung or of other pulmonary disease. A cooperative arrangement with Dr. Emerson Day, Director of the Kate Depew Strang Prevention Clinic of Memorial Hospital, and the active participation of two of its members, Drs. Genevieve Bader and Thomas Tamlyn, has made possible the procurement of ample material for this study as well as an experienced staff for its clinical evaluation. Sputum specimens collected at the Strang Clinic were sent to the "Papanicolaou Research Laboratory" of the Department of Anatomy, Cornell University Medical College, where smears were prepared, stained, and examined microscopically.

The material used for the second approach, the possible relationship of ciliocytophthoria to lung cancer or chronic pulmonary disease, consisted of specimens sent to the "Papanicolaou Cytology Laboratory" of the Department of Pathology, Cornell University Medical College, for a cytologic examination, chiefly from patients with obscure clinical symptoms involving a suspicion of cancer. Smears from these specimens

[1] From the Papanicolaou Research Laboratory, Department of Anatomy, Cornell University Medical College, New York 21, New York.

[2] This work was aided by a grant from the American Cancer Society, Inc.

were examined for CCP in the research laboratory in conjunction with those of the Memorial Strang Clinic series.

The third approach, the study of the relationship of CCP to a viral etiologic factor, was carried out at the Rockefeller Institute by Drs. James G. Hirsch and Cynthia H. Pierce in a separate group of patients with acute and chronic pulmonary disease. The microscopic examination of the smears prepared from the specimens obtained from these symptomatic patients was made by Dr. Pierce. Some of these observations have been reported in a preliminary note published in the *Proceedings of the Society for Experimental Biology and Medicine* (2).

Material and Methods

Since the material used for the study reported here came from two different sources, it was separated into two distinct groups: material obtained from the Kate Depew Strang Prevention Clinic, Series *A*; and material from the Papanicolaou Cytology Laboratory (Diagnostic), Series *B*.

Series A: The material of this series consisted of specimens of sputum collected under the supervision of Drs. Tamlyn and Bader. The patients were selected at random from persons who presented themselves for periodic routine examinations. In addition to the examination, these selected patients were further subjected by the above doctors to a thorough clinical study, including a detailed questionnaire covering any previous pulmonary ailment, smoking and drinking habits, residential environment, and occupational conditions which might affect the lungs. A full chest roentgenogram and a cytologic examination of sputum for malignancy, in addition to the examination for ciliocytophthoria, were also done in order to further document the cases used in this special project.

As a rule, two or three types of specimens were acquired in each case: an initial specimen taken at the clinic; the first sputum specimen produced the following morning, and a specimen obtained during the first acute upper respiratory infection, if any occurred after the original physical examination. All specimens were coughed directly into a jar containing approximately 40 ml. of 70 per cent ethyl alchol.

One thousand two hundred and seventy-eight sputum specimens were collected from a total of 556 persons. Fifty-six persons were excluded because they produced only non-deep cough specimens. The number of cases included in this series was, therefore, reduced to 500. Special instruction in postural position and exertion given by the clinician was the only aid used to induce a deep cough. The presence of carbon-laden macrophages in the smear was the main criterion used for accepting a specimen as satisfactory.

Four smears were prepared from each sputum specimen, using a mild crushing technique for spreading the sputum on the slides. These smears were fixed immediately in equal parts of ether and 95 per cent alcohol and stained after the Papanicolaou method, which was slightly modified in order to intensify the acidophilic reaction of cilia and the cytoplasmic inclusions of CCP forms.[3]

Series B: Material for the second part of this study consisted of smears from sputum specimens referred to the diagnostic cytology laboratory by hospital or private physicians for the purpose of excluding the presence of malignant cells in symptomatic cases of questionable pulmonary diagnoses. These smears, four from each specimen, had been processed and stained with the standard Papanicolaou procedure. They also had been examined in the diagnostic laboratory, and the cytologic findings were reported on the basis of the 5-class system, indicating the presence or absence of evidence for cancer.[4]

A total of 500 such cases, with satisfactory specimens and histories, taken in chronologic order, beginning with the year 1956, were used. These 500 cases represented 811 specimens, primarily from patients of the New York Hospital. Their charts were abstracted and thoroughly scrutinized for pertinent information by Drs. Richard Silver and Neda Voutsa. Histories of those patients whose specimens were sent to the laboratory by private doctors were obtained from the patient's own physicians.

Observations

Evaluation and Tabulation of Cellular Patterns Characteristic of Ciliocytophthoria

As already stated, the smears from both Series *A* and *B* were screened for cells indicative of ciliocytophthoria at the Papanicolaou Research Laboratory of the Department of Anatomy. Two smears from each case were examined by two screeners, each of whom examined one slide for twenty minutes under oil immersion (×1500). Their findings were tabulated on the basis of 38 selected varieties

[3] Hematoxylin (Standard Harris formula with glacial acetic acid), ¾ minute. NH4OH, 1.5 per cent solution in 70 per cent alcohol, 1 minute. OG6, 1½ minutes. EA65, 3 minutes.

A more detailed description of this staining procedure is given in a memorandum distributed with the second supplement of the *Atlas of Exfoliative Cytology* (3). Copies will be forwarded on request.

[4] Class I: Absence of atypical or abnormal cells
Class II: Atypical cytology but no evidence of malignancy
Class III: Cytology suggestive of, but not conclusive for, malignancy
Class IV: Cytology strongly suggestive of malignancy
Class V: Cytology conclusive for malignancy

of characteristic CCP forms. The smears of the *A* series were, in addition, examined for metaplastic and malignant cells under ×150 and ×600 magnifications.

The criteria used for the evaluation and tabulation of the 38 ciliocytophthoria varieties may be subdivided into three major groups: (*1*) those related to structural aberrations observed in the nucleus; (*2*) those related to alterations in the structure of the cytoplasm; and (*3*) those denoting a process of disintegration of the cell as a whole.

1. Changes affecting the nucleus: These may be subdivided into two main categories: one consisting of cells with pyknotic, deeply stained nuclei (figures 2, 15); and the other, of cells exhibiting a disruption of the normal nuclear pattern and the distribution of the chromatin into clumps, often arranged peripherally inside the nuclear membrane (figures 14, 19, 21). Each of these two types appeared in a variety of forms. In some instances, the whole pyknotic nucleus showed a change in its staining reaction comparable to that of the inclusion bodies described below (figures 26–28).

2. Changes related to the cytoplasm: A very prominent feature of the ciliocytophthoria process is the appearance of acidophilic inclusion bodies within the cytoplasm of the degenerating ciliated cells, many of which have been deprived of their cilia (figures 2, 5, 13, 19). These acidophilic bodies may vary greatly in size and number in any given cell and are sometimes seen in close proximity to the nucleus or occasionally within the nucleus itself, intimately connected with the chromatin complex (figures 17, 18, 19, 22, 23). Their origin is still somewhat obscure. The close contact of some of these bodies and their occasional presence within the nucleus suggest the possibility that they represent particles of chromatin undergoing a chemical change. As for their nature, it might be tempting to interpret them as inclusion bodies comparable to those observed in cases of viral infections, a view which finds strong support in the present observations.

Intracellular acidophilic inclusions were observed by Bryan and Bryan (4–6) in their painstaking studies on ciliated cells found in smears prepared from nasal mucous secretion. These writers noted that, in many persons afflicted with common cold (apparently of viral origin), the smears contained ciliated cells exhibiting characteristic acidophilic inclusions. They also observed marked degenerative changes in the ciliated cells, both cytoplasmic and nuclear, such as the pinching off or fragmentation of these cells and the peripheral clumping of chromatin within the nucleus.

Pigarevski in a recent paper (7) expresses the opinion that the presence of acidophilic bodies represents a defense reaction against the influenza virus. According to him, the acidophilic inclusions seen in the ciliated cells in tissue sections of the bronchial mucosa of mice which have been infected with influenza virus contain microcolonies of the virus. His concept is that these inclusions act as carriers for the removal of the virus from infected cells, which may undergo either total or partial disintegration. According to Pigarevski, in the latter case, the remaining nucleated basal portion may regenerate into a new ciliated cell.

3. Fragmentation and disintegration of the ciliated cell: One of the most marked cellular alterations resulting from the ciliocytophthoria process is the fragmentation or the complete disintegration of the ciliated cell. Fragmentation results in the separation of the cell into two parts (figures 1–3), a distal anucleated ciliated tuft (figures 6–9) and a proximal nucleated portion (figures 10, 11). Disintegration of the cell may sometimes be complete, resulting in a dispersion of the cytoplasm into amorphous masses of cellular detritus and free acidophilic inclusion bodies.

In cases of proved carcinoma of the lung, some of the above described cytoplasmic and nuclear degenerative changes are seen in cells exhibiting malignant characteristics.

Discussion of Statistical Data

Series A

The patients of Series *A* were essentially asymptomatic when they appeared at the Clinic for routine examination. For statistical purposes, the cases of Series *A* were divided into six separate groups, depending upon their clinical status at the time the specimen was obtained:

1. Normal (162 patients): Initial examination specimens and repeat morning specimens from patients having totally negative clinical findings, a negative chest roentgenogram, and a Class I cytologic report. No patient who developed an

acute upper respiratory condition within three months was included in this group.

2. Preacute (51 patients): Initial and morning specimens from patients similar to those in *Group 1* but who developed acute upper respiratory conditions within a rather arbitrary interval of three months following the initial or morning specimen.[5]

3. Chronic (202 patients): Initial and morning specimens from ambulatory patients having clinical findings of a mild pulmonary, chronic inflammatory condition or having negative clinical findings but a Class II cytologic evaluation. No patient who developed an acute upper respiratory condition within three months was included in this chronic group.

4. Preacute with underlying chronic conditions (64 patients): Initial and morning specimens from patients similar to those in *Group 3* but who developed acute upper respiratory conditions within three months following the initial or morning specimen.[5]

5. Acute (83 patients): Specimens collected during an acute upper respiratory infection, chiefly common colds, from patients without evidence of underlying chronic inflammatory conditions.

6. Acute with underlying chronic conditions (82 patients): Specimens collected during an acute upper respiratory infection from patients who had evidence of mild chronic inflammatory conditions at the time of the initial examination.

To tabulate the ciliocytophthoria findings in the above mentioned six clinical groups, the 38 CCP varieties were grouped into three general types of criteria: *T*—tufts of all types, with or without inclusions; *P*—CCP forms exhibiting pyknosis of the nucleus with or without cytoplasmic inclusions; and *C*—CCP cells with characteristic chromatin clumping with or without cytoplasmic inclusions.

In order to evaluate quantitatively the findings in the various clinical groups, the average number of *T*, *P*, and *C* cells per specimen in each group was first computed. A strict comparison of the average number of *T*, *P*, and *C* cells in each clinical group would, however, be impractical due to the occasional presence of extreme variation in some individual cases. In order to minimize the effect of these variations, the average number of cells found in the normal group was selected as a control, and the evaluation of the other clinical groups was based on the percentage of patients having *T*, *P*, or *C* findings above the average of the normal group. These percentages are listed in table 1 and illustrated in text figure 1.

TABLE 1

PERCENTAGE OF CASES PER CLINICAL GROUP WITH T, P, AND C FINDINGS ABOVE THE NORMAL AVERAGE

Clinical Group	Number of Cases	Percentage of Cases		
		Tufts	Pyknosis	Clumping
1. Normal	162	29.63	32.10	29.01
2. Preacute	51	31.37	33.33	37.25
3. Chronic	202	47.03	45.54	49.01
4. Preacute with chronic	64	43.75	51.56	46.88
5. Acute	83	49.40	54.22	54.22
6. Acute with chronic	82	63.41	51.22	54.88
Combined inflammatory (Groups 3, 4, 5, and 6)	431	50.58	49.19	50.81

[5] It should be noted that many of the patients who contributed pre-acute specimens, tabulated in *Groups 2* and *4*, were again represented in *Groups 5* and *6*, when specimens were collected during an acute upper respiratory condition.

PLATE I

CILIATED CELLS EXHIBITING SIGNS OF CILIOCYTOPHTHORIA (CCP)
(Magnification × 1800) (*see* text, pp. 643)

Cells showing changes affecting the nucleus, such as pyknosis (figures 2, 15, 16), distribution of the chromatin into clumps arranged peripherally inside the nuclear membrane (figures 12, 14, 19–21), or fading of the nucleus and change in its staining reaction comparable to that of the inclusion bodies (figures 27, 28).

Changes related to the cytoplasm, such as presence of acidophilic inclusion bodies (figures 2, 5, 13, 16, 19, 20), proximity of the acidophilic bodies to the nucleus, or direct contact with the chromatin complex within the nucleus (figures 17–19, 22, 23).

Fragmentation and disintegration of the ciliated cell (figures 1–5, 24, and 25), separation of the cell into a distal anucleated ciliated tuft (figures 6–9), and a proximal nucleated portion (figures 10 and 11).

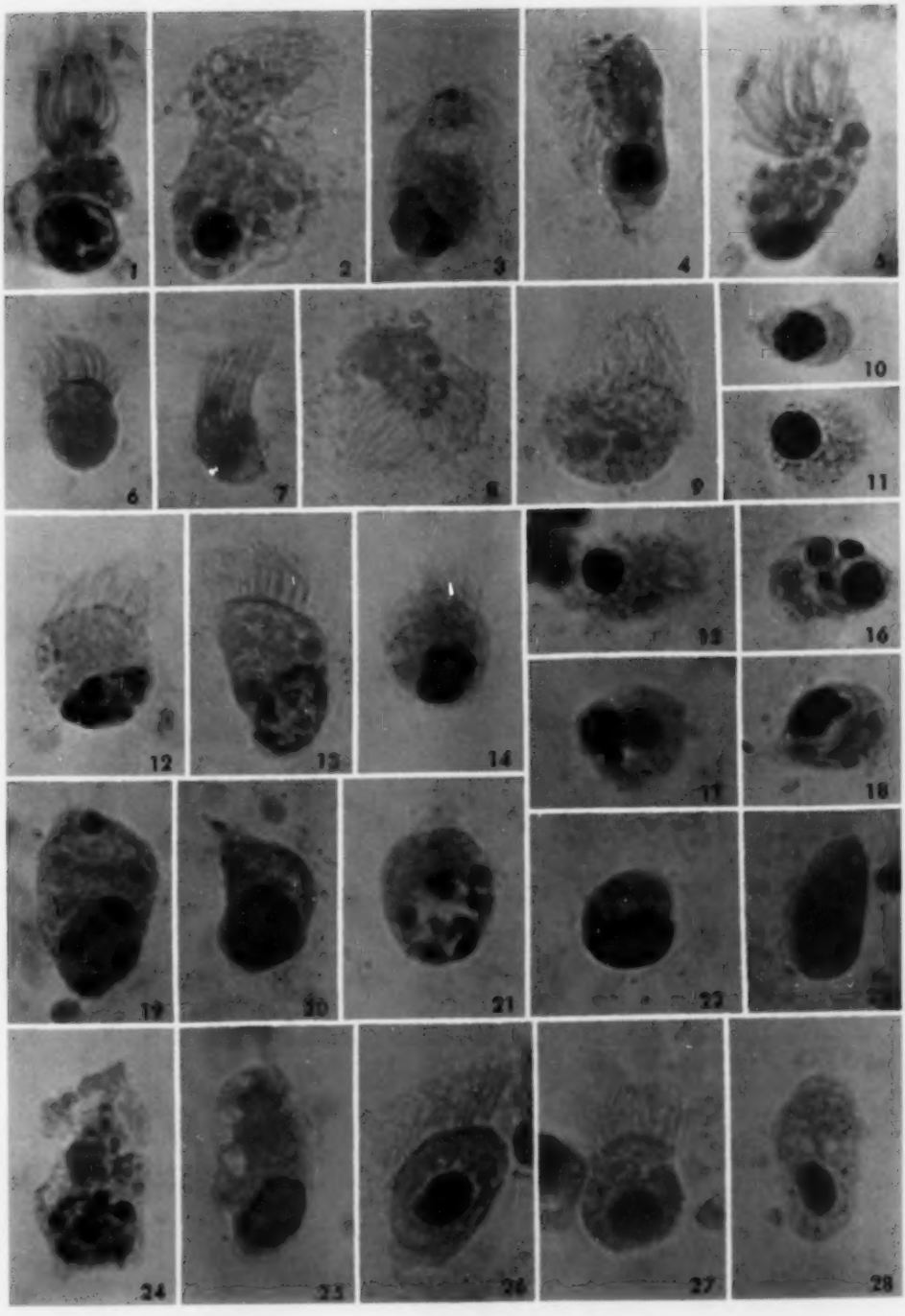
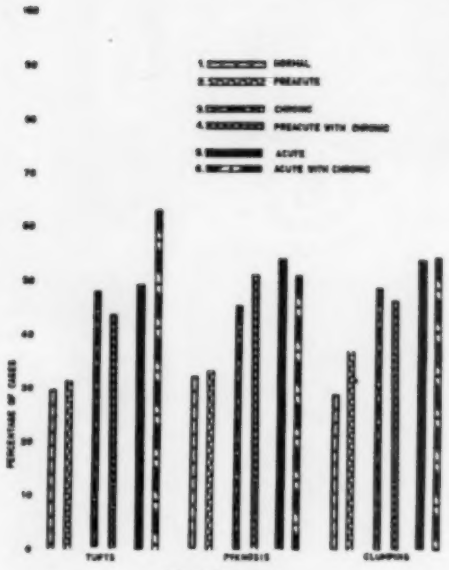


PLATE I



Text figure 1

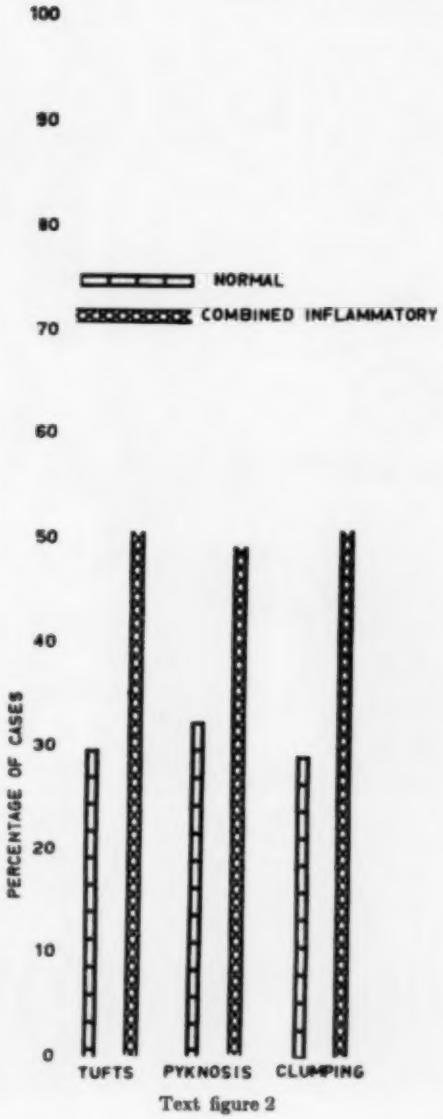
Text figure 1 indicates that the percentage of cases with ciliocytophthoria findings above the normal average increases from the normal to the preacute and chronic and reaches its highest level in the acute groups.

By combining clinical *Groups 3, 4, 5,* and *6* into one category, it is possible to demonstrate more clearly the differences in the percentages above the normal average in the normal group and those of a combined group consisting of all patients presenting inflammatory symptoms (text figure 2).

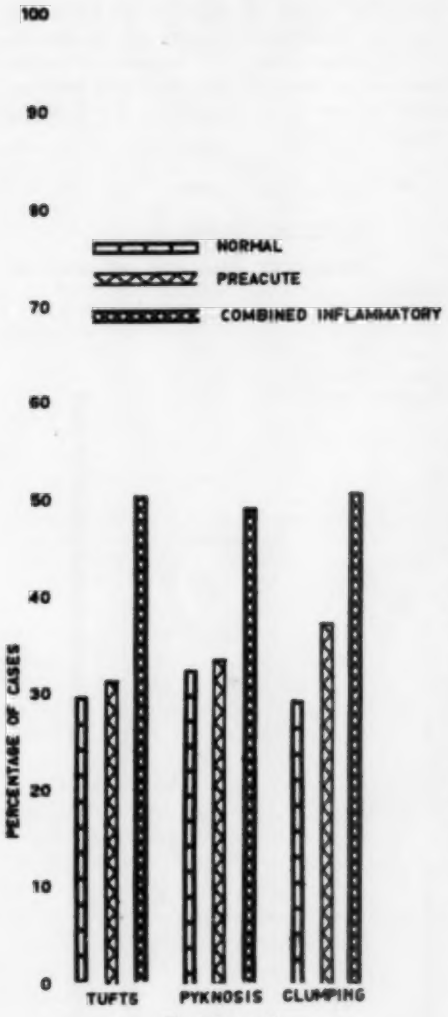
It is of interest that the preacute group (*Group 2*) representing patients who, although normal at the first examination, developed symptoms of acute inflammatory conditions within a period of three months, occupies an intermediate graph position between the normal group and that of combined inflammatory cases, as seen in text figure 3.

Another method for evaluating the findings statistically was to combine quantitative and qualitative values by taking into account not only the presence but also the number of individual varieties of *T, P,* and *C*. The total number of CCP cells found in a specimen, regardless of type, was multiplied by the number of varieties found in the same specimen in order to establish a "cyliocytophthoria numerical index" for a given case. In the cases in which more than one specimen was available, the procedure followed in this as in the previous tabulations was to give preference to the specimen which provided the highest CCP reading.

This index method was a result of the realiza-



Text figure 2



Text figure 3

tion that, in the majority of cases, the number of varieties present increases in direct relation to the total number of CCP cells. Whether this is due to a developmental sequence or to adequacy of sampling, the "CCP numerical index" assists in giving a proportional value to occasional patients who showed several varieties but a relatively small total number of CCP cells.

The percentage of patients in each diagnostic group who had a CCP numerical index less than 100 and the percentage of patients in whom the index was 100 or greater are shown in table 2. This separation appeared to be justified on the basis of the numerical index frequency distribution graph (*see* text figure 7), in which the highest percentage of cases tabulated per 100 index units was found to be in the first hundred. This percentage peak was followed by a definite drop in each group of cases which, however, varied in degree according to the clinical diagnosis.

A graphic illustration of table 2 is given in text figure 4.

The section on the left shows the percentage of cases in each clinical group with a numerical index below 100; and the section on the right, the percentage of cases in the same groups with a numerical index of 100 or more. In the low index section, there is a gradual gradation from the higher percentage found in the normal group to the lower percentage in the groups with acute disease. A reverse trend is, of course, seen in the section illustrating the percentage of patients with an index of 100 or more. These results are in line with those shown in previous graphs based on a different statistical approach.

The inflammatory groups (*Groups 3–6*), combined into a single clinical group and compared with the normal group, are shown in text figure 5.

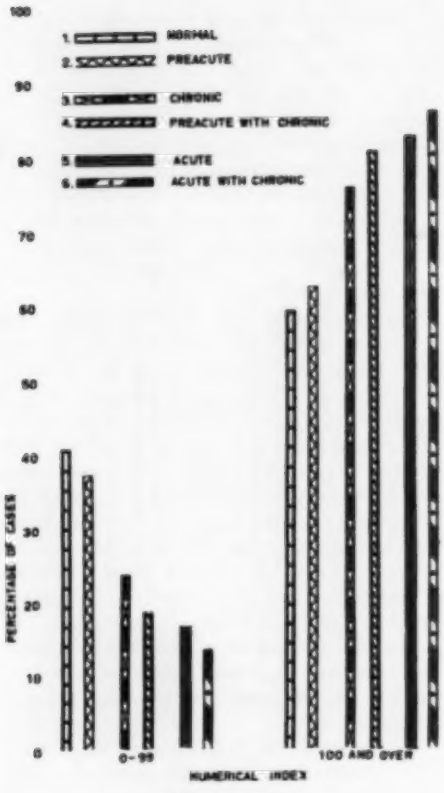
Text figure 6 adds to text figure 5 the preacute group (*Group 2*), which again assumes an intermediate position between the normal and combined inflammatory cases, although its variance from the normal group is not marked.

The frequency distribution of the three groups (normal, preacute, and combined inflammatory) are shown in text figure 7. Although the number

TABLE 2

DISTRIBUTION BY NUMERICAL INDEX

Clinical Group	Number of Cases	0 to 99	100 and More
		per cent	*per cent*
1. Normal	162	40.74	59.26
2. Preacute	51	37.26	62.75
3. Chronic	202	23.77	76.23
4. Preacute with chronic	64	18.75	81.25
5. Acute	83	16.87	83.13
6. Acute with chronic	82	13.41	86.59
Combined inflammatory (Groups 3, 4, 5, and 6)	431	19.72	80.27



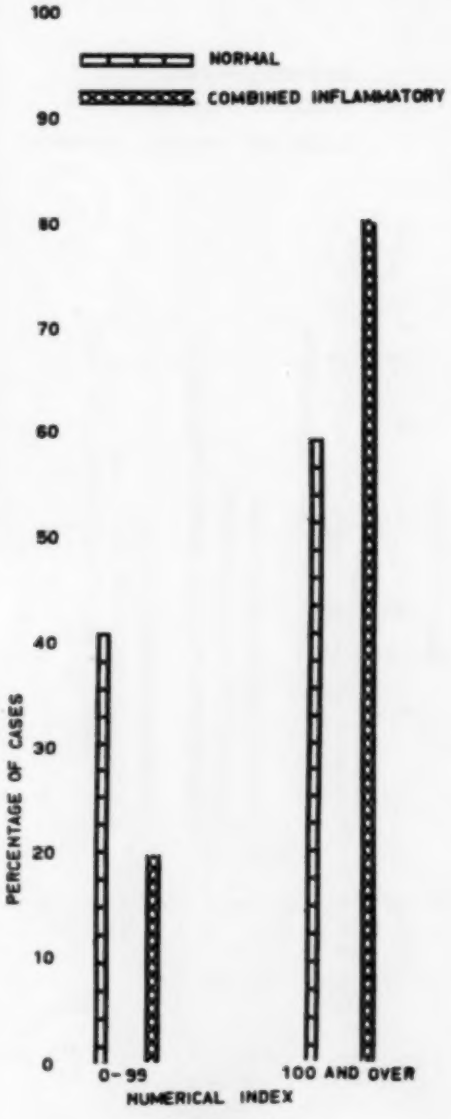
Text figure 4

of cases in each clinical group differs greatly, making it difficult to draw a strict comparison, it is evident that the highest frequency in any clinical group is in the first column, 0 to 99, with a sharp drop following immediately. This was the reason for using a numerical index of 100 as the dividing line in the preparation of text figures 4, 5, and 6.

It should be noted in text figure 7 that the bars showing the frequency distribution in the inflammatory cases are, in general, lower and spread over a wider range than both the preacute and the normal, respectively; however, occasional cases may be found at high index levels in all three groups.

Series B

The patients of Series B were essentially symptomatic. Their specimens were referred by hospital or private physicians to the Diagnostic Cytology Laboratory because of an obscure clinical picture involving a suspicion of cancer. In order to facilitate their statistical analyses, these patients were divided into the following diagnostic groups:



Text figure 5

Normal (37 patients): Patients having essentially negative pulmonary findings, a negative chest roentgenogram, and a Class I cytologic report.

Chronic (268 patients): Symptomatic patients having clinical and/or roentgenographic findings of a pulmonary, chronic inflammatory condition plus patients with negative findings but a cytologic report of Class II. It should be noted that these patients had severe enough symptoms to require hospitalization or the attention of a private physician. This group includes such conditions as: asthma, bronchitis, bronchiectasis, emphysema, tuberculosis et cetera.

Acute (70 patients): Patients having clinical and roentgenographic findings of an acute pulmonary disease (bacterial or viral pneumonia and bronchitis) and patients having acute conditions associated with underlying chronic inflammatory disease. These two types of acute cases were combined because of similar findings.

Malignant (70 patients): Patients proved to have primary pulmonary carcinoma.

In addition, there were 55 patients with various other conditions, none of which were sufficiently numerous to warrant a separate statistical analysis. These cases included bronchial adenomas, pulmonary emboli, and patients in whom the presence of a carcinoma was suspected but not proved.

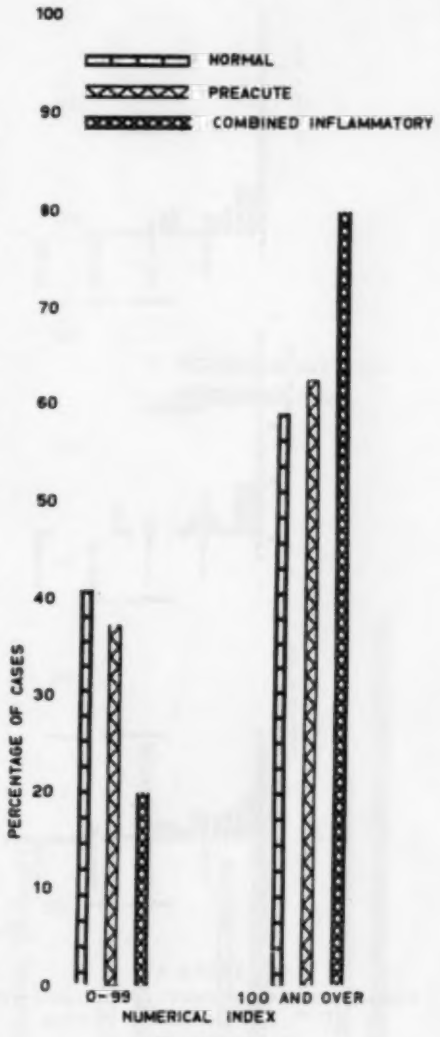
In a quantitative evaluation of Series *B*, the average number of ciliocytophthoria cells of each type per specimen in the normal group was selected as a basis for evaluating the other clinical groups, as previously done in Series *A* (see p. 654).

The percentage of cases in each clinical group with *T*, *P*, and *C* findings above that of the normal average is shown in table 3.

A graphic representation of the percentages given in table 3 for the normal, chronic, and acute groups is shown in text figure 8.

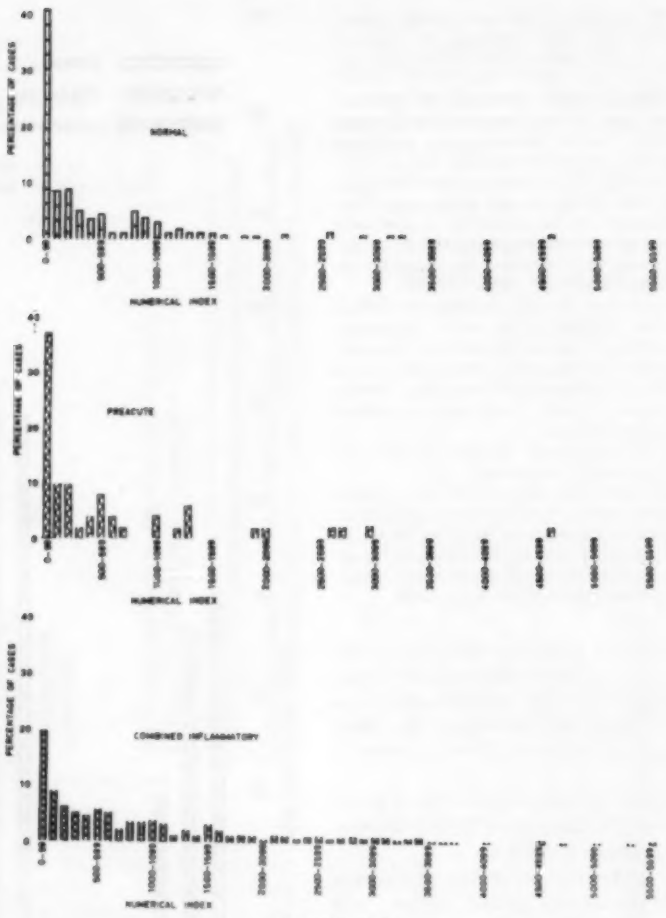
In text figure 8, a lower percentage is shown in the normal than in the inflammatory groups, chronic or acute. It should be noted, however, that, contrary to the findings in Series *A*, the percentages in the acute group for the *T* and *P* types are lower than those of the chronic group. This discrepancy might possibly be attributed to the fact that, in Series *B*, only 9 of 70 cases were diagnosed as being of viral etiology, whereas, in Series *A*, the great majority of the acute cases were presumably of viral origin since they were observed during the period of the influenza epidemic of 1957.

If the cases of the acute group of Series *B* are separated on the basis of their etiology, viral or bacterial, as suggested by the clinical findings, a difference in CCP percentages in favor of the viral group is apparent, as shown in text figure 9. It should, of course, be taken into consideration that the number of cases involved in this figure is relatively small.



Text figure 6

A combination of the chronic and acute groups into a single inflammatory unit is shown in text



Text figure 7

TABLE 3

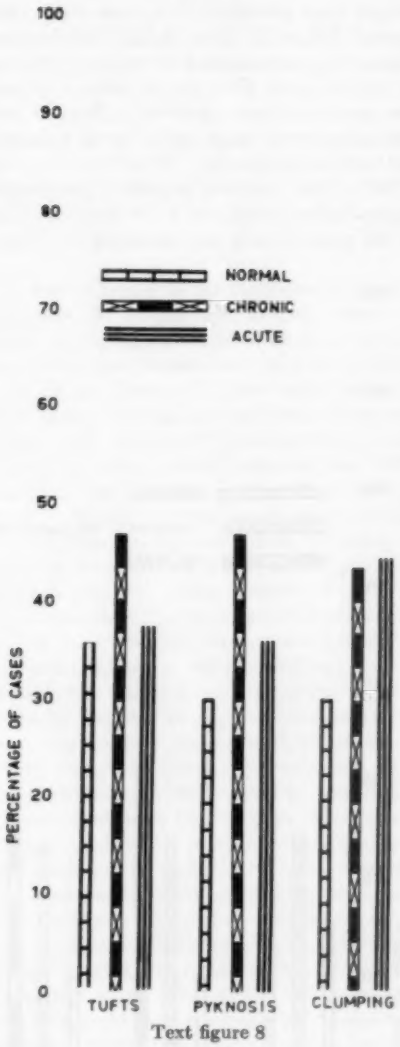
PERCENTAGE OF CASES PER CLINICAL GROUP WITH T, P, AND C FINDINGS ABOVE THE NORMAL AVERAGE

Clinical Groups	Number of Cases	Percentage of Cases		
		Tufts	Pyknosis	Clumping
1. Normal	37	35.14	29.73	29.73
2. Chronic	268	46.64	46.64	43.28
3. Acute	70	37.14	35.71	44.29
Combined inflammatory (Groups 2 and 3)	338	44.67	44.38	43.49
4. Malignant lung, primary	70	47.14	51.43	48.57

figure 10, in which the percentages in the inflammatory group are higher than those in the normal group. The ascending trend evidenced in text figure 10 is further extended by the addition of the percentage findings in the malignant group which reaches an even higher level, as shown in text figure 11.

The tabulation of CCP findings in Series *B* on the basis of the previously described CCP numerical index (page 647) are shown in table 4 and illustrated in text figure 12.

In text figure 12 the percentage of cases with a CCP numerical index below 100 follows a descending trend from the normal to the combined inflammatory and malignant groups in contrast

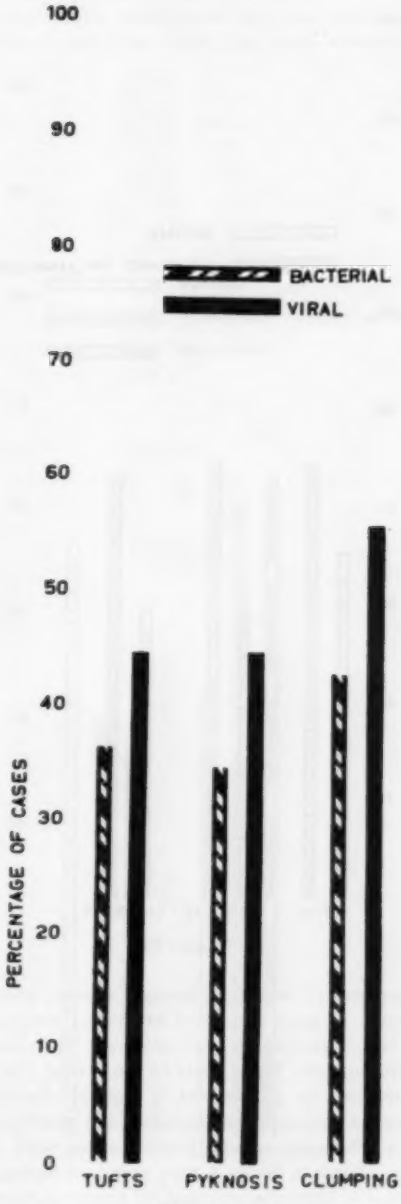


Text figure 8

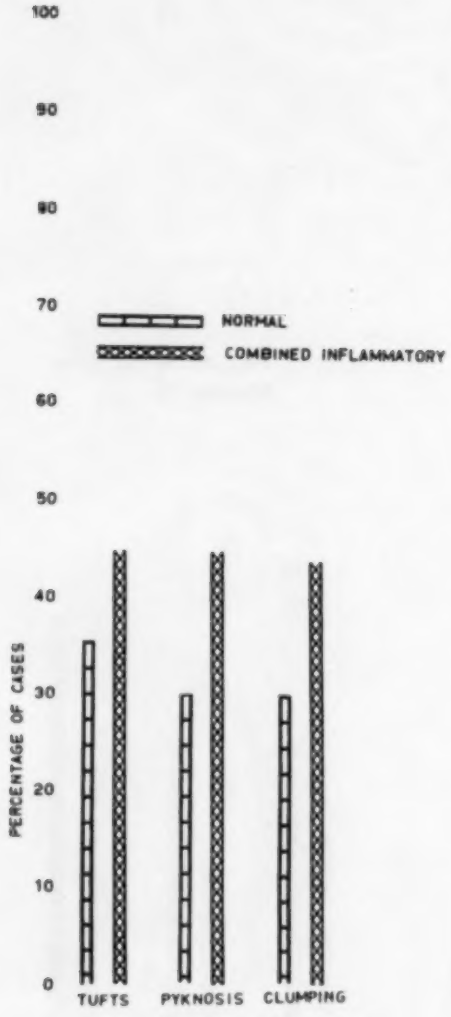
to those having an index of 100 or more in which the trend is reversed.

Discussion

Before attempting to analyze the statistical results of Series *A* and *B*, certain basic differences between these two groups should be reviewed and given due consideration. One of the differences consists in the fact that the patients in Series *B* were largely symptomatic, with a pre-



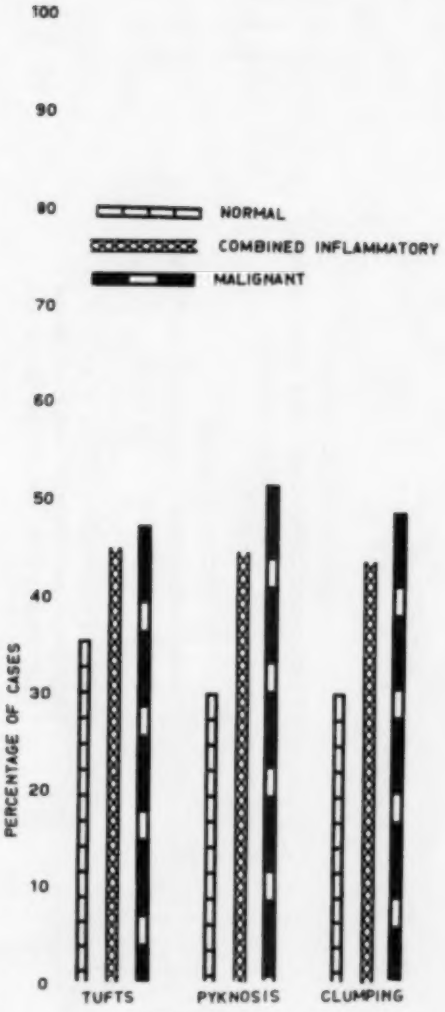
Text figure 9



Text figure 10

dominance of chronic pulmonary disease, severe enough to require medical attention; whereas, in the *A* Series, the patients were essentially asymptomatic. Their visits to the Strang Clinic were for the purpose of a general checkup directed primarily to excluding the possibility of a subclinical cancer. However, since many of the patients in Series *A* were examined during a period of time characterized by recurring waves of viral epidemic, a fair percentage of them developed acute pulmonary symptoms within three months following their initial examinations. These symptoms consisted of attacks of influenza or common colds, while the majority of patients with acute symptoms observed in Series *B* were hospitalized with diagnoses of acute bronchitis and bacterial pneumonia.

The original plan was to make a comparative study of ciliocytophthoria in two separate groups of 500 patients each, one consisting of sympto-



Text figure 11

TABLE 4

DISTRIBUTION BY NUMERICAL INDEX

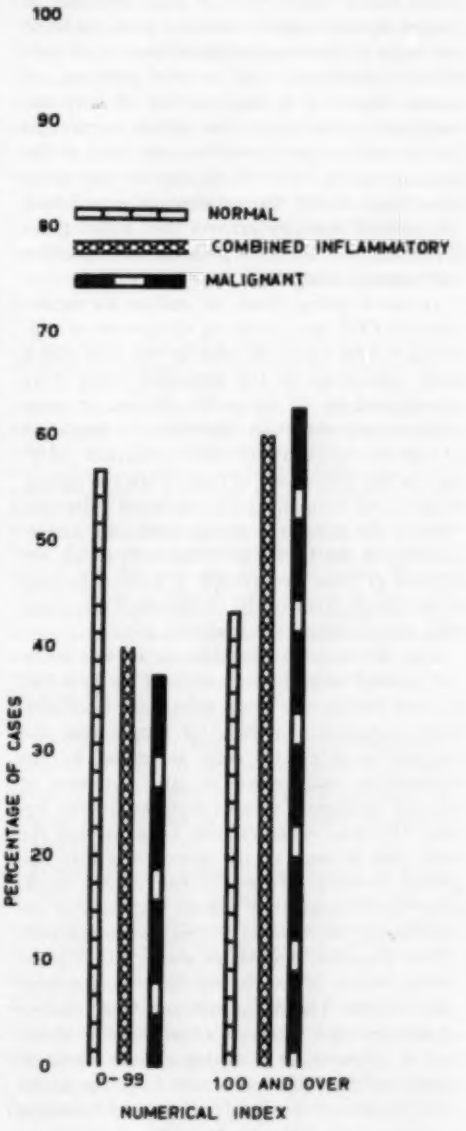
Clinical Groups	Number of Cases	0–99	100 and more
		per cent	*per cent*
1. Normal	37	56.76	43.24
Combined inflammatory (Groups 2 and 3)	338	39.94	60.06
4. Malignant lung, primary	70	37.14	62.86

matic cases referred to the Diagnostic Cytology Laboratory for examination and the other of normal, asymptomatic cases to be used as controls. It was thought that material from patients at the Strang Memorial Clinic would be eminently suitable for the control group. However, the previously mentioned recurring waves of an influenza epidemic greatly affected the CCP readings in the *A* Series. It was thus realized that the "Memorial" group could not be used as a normal standard for comparison. However, it proved to be rewarding from another point of view as it provided strong evidence in favor of a close relationship between the CCP phenomenon and viral infections. Such a relationship is fairly well indicated in text figures 2 and 5.

This point was further elaborated by the Rockefeller Institute group, who have been able to correlate the CCP findings with the presence of virus by special laboratory procedures (2). They found that, in thirty cases of viral influenza, twenty-six were CCP positive. In contrast, CCP was found rarely in sputum from patients with pneumonia or with other chronic respiratory diseases of bacterial origin. They thus concluded that "sputum cytology may prove valuable for prompt differential diagnosis between viral and bacterial respiratory infections."

In a more recent paper (1960), "Ciliocytophthoria in Sputum from Patients with Adenovirus Infections," Pierce and Knox (8) state that the results of their studies "demonstrate the association of CCP with both adenovirus and influenza disease and support the concept that CCP may occur in a variety of viral respiratory infections."

Another point of interest brought to light by this study was the fact that, in the group of the originally clinically negative cases of Series *A*, a secondary group could be identified in which acute symptoms of viral infections (common colds) appeared within three months. This group was separated and designated as *preacute* (*Group 2*). In this preacute category, the CCP readings were somewhat higher than in the other apparently normal cases, indicating that a virus infection had, in all probability, been present in a latent, subclinical form. This lends support to



Text figure 12

the view that a virus infection may often persist in a latent form without any apparent symptoms.

The examination of sputum for CCP thus offers a new approach to the study of this point which has some rather significant clinical and public health implications. A mass screening of sputum smears might eventually prove to be of real value in the recognition of latent viral pulmonary infections, just as the screening of vaginal smears is in the detection of early unsuspected carcinomas of the uterine cervix. The fact should be considered, however, that, in the final evaluation of CCP findings in any given case, there is still the problem of establishing well-defined objective criteria that would make it possible to draw a sharp line between positive and negative cases.

In the *B* series, efforts to analyze the significance of CCP as a factor in the genesis of cancer have been much impeded by the fact that a large percentage of the malignant cases were complicated by an associated chronic or acute inflammatory condition. However, an inspection of text figures 11 and 12 shows a marked difference in the percentage of cases when the normal is compared with either the combined inflammatory or the malignant group, indicating a relationship of the CCP phenomenon to either one or both of these two groups. A lesser difference is evident in favor of the malignant if compared with the combined inflammatory group.

One of the main contributions of this study has perhaps been the realization of the fact that in both chronic and acute pulmonary infections, more particularly in those of viral origin, the destruction of ciliated cells, as proved by the microscopic examination of large numbers of sputum specimens, reaches quite impressive figures. One may conservatively estimate that the daily loss in some of the acute cases may approach or even surpass the half million mark. It is thus reasonable to assume that such an extraordinary depletion of the ciliated components of the bronchial epithelium would, in all probability, result in the formation of naked nonciliated areas. The loss of such appalling numbers of cells during a whole period of an acute illness and of a sometimes persisting chronic condition might not be fully compensated by the active proliferation of the basal cells of the bronchial mucosa, particularly in persons of advanced age, whose tissues have a decreased regenerative potential.

Naked nonciliated areas are centers of disruption of the normal ciliary action, which performs the all-important function of the continual cleansing of the lumina of the bronchi. A. C. Hilding (9), whose ingenious experimental studies have done so much to clarify this point, showed that

> ...the mucous blanket in the tracheo-bronchial tree, when made observable by india ink, may be made to impinge upon an artificially deciliated island as upon an obstruction. It may remain for a matter of hours upon such a spot while ciliary action and streaming of mucus continues in surrounding areas....

In experimenting with cigarette tar, he also noted that accumulations of tar may be similarly produced in such naked areas by cigarette smoking.

In the light of these observations, it might be justifiable to draw the conclusion that acute and prolonged chronic pulmonary infections are very likely to cause a severe depletion of the ciliated components of the bronchial epithelium and the formation of deciliated islands, thus opening the gateway to the action of various extraneous carcinogenic factors. This may provide us with a key to explain the appearance of multiple islands of basal cell hyperactivity, metaplasia, and precancerous or early cancerous changes in the lungs of heavy smokers, which have been brought to light through the classical studies of Auerbach, Stout, and their co-workers (10–13). It may also serve as further evidence in support of the view of a multicentric origin of cancer.

In studies from this laboratory, the presence of metaplastic cells in smears strongly positive for ciliocytophthoria has repeatedly been observed. These data are, however, inconclusive in regard to their possible etiologic relationship to CCP or cancer, although in a few isolated cases a sequence of progressive changes has been noted which ended in the appearance of cancer. In one of these (14), sputum examinations, initiated because of suspicious clinical symptoms, were repeated periodically from September, 1955, when the first smear was taken, through December, 1956. The patient was seventy years old and a heavy smoker. Examination of the original sputum smears showed typical CCP forms

which persisted in varying numbers in subsequent smears.

During the first seven months, metaplastic and other atypical cells were also seen in the smears but did not arouse a suspicion of malignancy until July, 1956, when some of the metaplastic cells began to display distinctly abnormal features. A smear containing conclusively malignant cells was first obtained in October, 1956. An exploratory operation performed in December, 1956, largely on the basis of the cytologic findings, revealed the presence of a very early epidermoid carcinoma measuring about 6 by 3.5 by 2.5 mm. The patient has fully recovered from a right upper and middle lobe lobectomy and, four years after the operation, is still enjoying good health and continuing his regular professional duties. The clean-cut and well-documented sequence of CCP, metaplasia, and carcinoma of the lung in this case is indeed very suggestive but, of course, insufficient to prove the validity of an etiologic relationship.

Further light was thrown upon this case by the findings of Dr. Oscar Auerbach who has kindly extended to us his cooperation by studying the bronchial mucosa of the two excised lobes of the right lung with his painstaking technique of step serial sections of the entire specimen. In the 46 blocks included in Dr. Auerbach's examination he was able to observe 4 epithelial lesions suggestive of metaplasia and 6 others interpreted as carcinomas *in situ*.

A relationship of chronic and acute inflammatory infections to a subsequent appearance of cancer has also been advocated by a number of clinical investigators, who provided good statistical evidence in favor of this view. Case and Lea (15) found that deaths due to cancer of the lung among 1,421 British World War I pensioners, who were known to have bronchitis at the time of imprisonment, were at twice the rate of that of the general population. Walter Finke (16) states that an

> ... analysis of the hospital records of 159 patients with proved primary lung cancer revealed that, in one third of these cases, a long standing pulmonary ailment had existed.... The results of this study support the view that chronic pulmonary disease may be one of the 'nearer causes' of bronchogenic carcinoma.

This would support other observations which point toward chronic, postinflammatory processes as a possible etiologic factor in lung cancer. "Of such lung damaging illnesses, the most frequent," according to Finke (17), "proved to be influenza or pneumonia, during the epidemic of 1918–20, which was proved to be of viral origin and in pneumonia at some other time in the patient's earlier life."

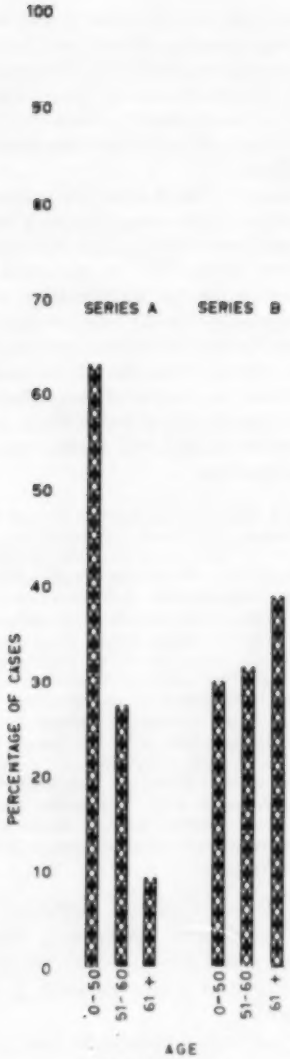
In some cases in which consecutive specimens included sputum taken during the acute stage of viral infection, it was evident that the high CCP figures shown at the time of the acute stage dropped soon after the disappearance of the clinical symptoms. Similar observations have been reported by the Rockefeller Institute group, which has followed a number of patients with viral infection over a period of time. This seems to be the general rule although there are instances in which the high CCP readings may continue for a long time.

One, which may be cited here, is that of a middle-aged woman, who, while visiting France, contracted a severe acute viral infection of Asian influenza. Examination of sputum specimens showed an unusually large number of degenerated ciliated cells (CCP). The acute condition was followed by a prolonged chronic stage, during which the CCP numerical index remained at an unusually high level. The high CCP reading persisted even after the complete disappearance of subjective symptoms. However, her husband, to whom she transmitted the disease soon after her return to the United States, showed a marked drop in the CCP count in his sputum specimens following the termination of the acute stage. This might have been the result of a variation in host reaction or of some other factor, such as a decrease in the virulence of the virus.

Another factor which has apparently contributed to some of the discrepancies noted between Series *A* and *B* has been the difference in the age distribution in the two series, as shown in text figure 13.

It may be seen in text figure 13 that, in Series *A*, the percentage of patients fifty years old or younger was 63.53 in contrast to that of Series *B*, which was 29.93. On the other hand, the percentage of patients aged fifty-one to sixty was 27.25 in Series *A* against 31.29 in Series *B*, and that of patients sixty-one and older was only 9.22 in Series *A* against 38.78 in Series *B*.

A computation of the average numerical index for each of the three age groups (0–50, 51–60, and 61-plus) in Series *B*, including all specimens

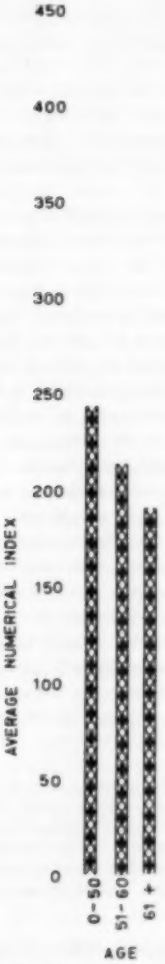


Text figure 13

obtained from each patient, is illustrated in text figure 14.

An average numerical index of 244.97 in the 0–50; 230.93 in the 51–60; and 192.02 in the 61 and older group is shown in text figure 14. These data indicate a trend toward lower CCP readings with increasing age.

An analysis of the CCP findings in relation to the extent of cytologic evidence of malignancy in the 70 cases of primary carcinoma showed that in the cases with more conclusive cytologic evidence, such as those of Class *V*, the CCP numerical index was relatively low when compared with that of the cytologically less conclusive cases. Assuming that a high cytologic evidence corresponds to a more advanced stage of malignancy, it might be presumed that the more profuse exfoliation of malignant cells, including many degenerated and necrotic forms, which might prevail in such cases, as well as the often markedly increased number of inflammatory



Text figure 14

cells resulting from secondary infections, would tend to mask many of the decidedly less conspicuous CCP cells.

Although the relative frequency of the three ciliocytophthoria types (*T*, *P*, and *C*), described on pages 642–643 and illustrated in Plate I, shows great variability, it might be stated in general that the tufts are more common findings than the cells exhibiting nuclear pyknosis or chromatin clumping. The significance and diagnostic value of each of these general CCP types, as well as that of their variant forms and their relation to viral infection or to pulmonary carcinoma, should be the subject of a more extensive and systematic study than could be undertaken at the present time. It is possible that such a study would contribute to the establishment of criteria that woud permit a more definitive interpretation and evaluation of the cytologic findings.

It is difficult to draw any definite conclusion at this time regarding the possible significance of the sex factor in the evaluation of CCP. It may, however, be stated that the ratio of males to females in the two series was comparable (79.97 per cent to 20.03 per cent, respectively, in the *A* Series and 74.83 per cent to 25.17 per cent in the *B* Series). On the other hand, in the 70 primary carcinomas of the lung in Series *B*, the ratio was still higher in the male component (87.14 per cent to 12.86 per cent), as might be expected, since pulmonary carcinomas are known to be more prevalent in males than in females.

It is believed to be important for the cytologist who specializes in the examination of sputum smears for the diagnosis of cancer to have some knowledge of the cellular changes linked with the CCP phenomenon. The large number of necrotic cells, which may be present in cases of acute or prolonged chronic pulmonary infections, especially in patients with high CCP readings, might sometimes be misleading and result in false interpretations, since necrosis is a relatively common finding in smears from malignant cases.

Summary

Previous studies have shown that in certain cases of acute and chronic pulmonary diseases there is a mass degeneration of ciliated cells of the bronchial epithelium which can be detected by microscopy of sputum specimens. The present studies have been undertaken for the purpose of exploring further the extent and significance of this phenomenon ("ciliocytophthoria," or "CCP") in two groups of patients: 500 essentially asymptomatic (*A*), and 500 symptomatic (*B*).

In both groups there was a definite increase in the CCP numerical index in sputum specimens from the chronic cases, and more so in the acute inflammatory cases. The advent of an epidemic of Asian influenza during the period of the *A* series study resulted in a still greater increase in the CCP readings in a large percentage of sputum specimens, particularly in patients with acute respiratory symptoms, indicating a possible etiologic relationship of this phenomenon to pulmonary infections of viral origin.

The relatively higher readings in the patients with primary carcinoma of the lung as compared with those of the combined inflammatory group in series *B* suggests a relationship of this phenomenon to malignant disease as well. A case is described in which positive CCP findings were followed first by the appearance of metaplastic cells, and later, malignant cells. A subsequent operation proved the existence of an early epidermoid carcinoma of the lung.

The mass destruction of ciliated cells in both chronic and acute pulmonary infections is likely to result in the formation of nonciliated areas on the surface of the bronchial mucosa, thus opening the gateway to the action of extraneous carcinogenic and other injurious agents.

Sumario

La Degeneración de las Células Ciliadas del Epitelio Bronquial (Ciliocitoftoria) en Su Relación con las Neumopatías

Estudios anteriores han revelado que en ciertos casos de enfermedades pulmonares agudas y crónicas existe una degeneración masiva de las células ciliadas del epitelio bronquial que puede descubrirse con la microscopía de ejemplares del esputo. Los estudios actuales fueron emprendidos con el objeto de explorar más a fondo la extensión y la importancia de este fenómeno ("ciliocitoftoria" o "CCF") en dos grupos de enfermos: 500 esencialmente asintomáticos (*A*) y 500 sintomáticos (*B*).

En ambos grupos, hubo un aumento bien definido del índice numérico de CCF en los ejemplares de esputo procedentes de los casos crónicos, y aun mayor en los casos inflamatorios agudos. El advenimiento de una epidemia de influenza asiática durante el período del estudio de la serie *A* dió por resultado un aumento todavía mayor

en las lecturas de la CCF en un porcentaje elevado de los ejemplares de esputo, en particular en los enfermos con síntomas respiratorios agudos, indicando la posible relación etiológica de este fenómeno con las infecciones pulmonares debidas a virus.

Las lecturas relativamente más altas de los enfermos de carcinoma primario del pulmón, comparadas con las del grupo inflamatorio combinado de la serie *B*, señalan igualmente una relación de este fenómeno con la afección neoplásica. Se describe un caso en el que los hallazgos positivos de CCF fueron seguidos primero, de la aparición de células metaplásicas, y después, de células malignas. Una operación subsiguiente demostró la existencia de un carcinoma epidermoideo incipiente del pulmón.

Es probable que la destrucción masiva de las células ciliadas en enfermedades pulmonares tanto agudas como crónicas dé por resultado la formación de zonas no ciliadas en la superficie de la mucosa bronquial, abriendo así las puertas a la acción de agentes extraños carcinógenos y nocivos de otro género.

Resume

Dégénérescence des cellules ciliées de l'epithelium bronchique (ciliocytophtoria) et ses relations avec la maladie pulmonaire

Des études antérieures ont montré que dans certains cas de maladies pulmonaires aiguës et chroniques, il y a une dégénérescence massive des cellules ciliées de l'epithelium bronchique, qui peut être vue à l'étude microscopique des échantillons d'expectoration. Les études actuelles ont été entreprises dans le but d'explorer davantage l'étendue et la signification de ce phénomène ("ciliocytophtoria" ou "CCP") chez deux groupes de malades: 500 essentiellement asymptomatiques (*A*) et 500 symptomatiques (*B*).

Dans chaque groupe, il y avait une augmentation nette de l'index numérique de CCP dans les échantillons d'expectoration de cas chroniques, et plus aussi dans les cas d'inflammation aiguë. L'apparition d'une épidémie de grippe asiatique pendant la période d'étude des séries *A*, aboutit à une augmentation encore plus grande des lectures de CCP, dans un large pourcentage des échantillons d'expectoration, en particulier chez des malades ayant des symptômes respiratoires aigus, indiquant une relation étiologique possible de ce phénomène avec les infections pulmonaires d'origine virale.

Les lectures relativement plus élevées chez les malades ayant un carcinome primaire du poumon, comparées à celles du groupe avec inflammation associée dans les séries *B*, fait de même penser à une relation entre ce phénomène et la malignité. Les auteurs décrivent un cas dans lequel les données positives de CCP furent suivies en premier par l'apparition de cellules métaplastiques et, plus tard, par des cellules malignes. Une opération ultérieure prouva l'existence d'un carcinome épidermoïde du poumon, à son début.

La destruction massive des cellules ciliées à la fois dans les infections chroniques et aiguës, a probablement pour résultat la formation de zones non-ciliées à la surface de la muqueuse bronchique, ouvant ainsi la voie à l'action d'agents étrangers carcinogènes ou d'autres agents nuisibles.

Acknowledgment

The writers wish to express their appreciation to Frances Bradner, Joan Farrell, Mary Giuffre, Barbara O'Connor, Adele Reboul, and Kayla Scheer for their assistance in this study.

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OBSERVATIONS ON THE NATURAL HISTORY OF BRONCHIOLO-ALVEOLAR CARCINOMA[1]

Experience with Twenty-One Cases

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Introduction

Contradictory views exist among pathologists regarding the natural history of bronchiolo-alveolar carcinoma. Disagreement over unicentric or multicentric origin and the mechanism of pulmonary dissemination has resulted in an uncertain rationale for surgical treatment. Some surgeons regard early bronchiolo-alveolar carcinoma as giving the most favorable prognosis of any pulmonary malignancy (1–3), while others have had contrary experience (4–6).

Isolated reports of unusual cases or large collected series often lack the coherence of detail seen in the evaluation of a small group. Experience with 21 patients with bronchiolo-alveolar carcinoma is presented with regard to clinical detection and factors influencing the length of survival. An attempt is made to relate the survival period to the distribution and duration of disease and to evaluate the results of surgical therapy.

Material and Methods

In an eight-year period (1950–1958), 21 cases were seen at this institution among 261 cases of bronchogenic carcinoma, an incidence of 8 per cent. The diagnosis in each case was established by histologic examination of tissue obtained at surgical biopsy, resection, or necropsy.

Observations

Clinical features: The average age for the group was 57.7 years, similar to the median age for bronchogenic carcinoma in general. The youngest patient was 34 years old, while the oldest was 77 years.

Symptoms and signs: Four patients in the group were asymptomatic (tables 1 and 2). In each, the pulmonary shadow had been detected on a routine survey film. Ten, or approximately half, of the patients presented with multiple pulmonary symptoms. For the most part, these symptoms were indistinguishable from bronchogenic carcinoma with secondary pneumonitis. A surprising observation was the early manifestation of neurologic signs and symptoms in 6 patients, or almost one-third of this small group. Several of these patients had moderately advanced pulmonary lesions which were as yet asymptomatic upon admission.

Almost all of the group with moderate and far advanced pulmonary involvement had many chest complaints, of which cough was the most common. Four patients were found to have heavy coughs productive of excessive mucoid or foamy sputum thought to be pathognomonic of this mucus-producing tumor. Three patients had pulmonary osteoarthropathy.

Marked weight loss was a common symptom; in one patient, up to 44 pounds in six months. In fact, such complaints as malaise, weight loss, shortness of breath, and night sweats were not unlike those attributed to active tuberculosis.

Roentgenographic findings: Four of the 21 patients presented with discrete so-called coin lesions in the peripheral lung field (figure 1*A*, *B*). In another 5 patients, the tumor mass failed to be well circumscribed and usually exceeded 2 to 3 cm. in diameter. By far the commonest roentgenographic pattern in 12 of the patients was a diffuse, rather ill-defined segmental or lobar pneumonia (figure 1*C*). This type of infiltration was associated with a pleural effusion in 4 patients and apparent cavity formation in 2 patients. The resemblance of this chronic pneumonitis to active pulmonary tuberculosis led to diagnosis and treatment of the latter disease in 3 cases.

The pattern of onset of this illness as manifested in serial roentgenograms was equally nonstereotyped. In 7 patients, each having had normal annual survey films, there was an abrupt appearance of either a well-delineated coin lesion or a fairly large, dense, "inflammatory"

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TABLE 1

BRONCHIOLAR CARCINOMA-SURGICAL TREATMENT

Patient	Signs and Symptoms on Admission	Operation	Survival	Status
1. J.J.	Cough and dyspnea	Pneumonectomy	9 years	Living and well
2. F.W.	Cough, hemoptysis, Horner's syndrome, and brachial plexus pain	Lobectomy and X-ray treatment	3 months	Died
3. A.L.	Neurologic complaints	Lobectomy	4 months	Died
4. J.H.	Neurologic complaints	Pneumonectomy	4 months	Died
5. H.B.	Asymptomatic	Pneumonectomy	5 years	Died
6. H.L.	Cough, weight loss, fatigue	Pneumonectomy	3 years	Died, coronary thrombosis
7. A.W.	Neurologic complaints	Lobectomy	1 year	Died
8. P.W.	Asymptomatic	Lobectomy	2 years	Died
9. P.S.	Pleurisy and cough	Lobectomy	1 year	Died
10. C.S.	Asymptomatic	Lobectomy	3 years	Living and well
11. A.D.	Cough and hemoptysis	Pneumonectomy	2 years	Living and well
12. B.D.	Neurologic complaints and cough	Pneumonectomy	2 days	Died, pulmonary edema and emboli

TABLE 2

BRONCHIOLAR CARCINOMA-NONSURGICAL TREATMENT

Patient	Signs and Symptoms on Admission	Diagnosis Established	Treatment	Survival
1. J.C.	Cough, weight loss, pleural effusion	Postpleural effusion	Nitrogen mustard	1 week
2. G.C.	Neurologic complaints	Post supraclavicular node	None	6 weeks
3. J.L.	Chest pain, cough, dyspnea	Post supraclavicular node	Intrapleural P32	4 months
4. T.B.	Dyspnea, cough	Postbronchial biopsy	None	1 month
5. A.N.	Cough, dyspnea	Postpleural effusion	None	1 month
6. A.L.	Chest pain, cough, dyspnea	Exploratory thoracotomy	X-ray, 7,000 r	2 years
7. W.H.	Chest pain, pleurisy, dyspnea	Lung biopsy (diagnosis questionable)	None	5 years
8. T.C.	Bone pain from Paget's Disease	Diagnosis not established ante mortem	None	1 month
9. R.B.	Neurologic complaints, cervical mass	Postcervical and axillary node	X-ray to shoulder, 2,100 r	9 months

mass from 6 to 8 cm. in diameter. The sudden appearance of a segmental or lobar infiltration suggested pneumonia to the observers, and only upon nonresolution of the shadow was the carcinoma suspected. On the other hand, the smaller coin lesions were often circumspect from their first observation. All lesions in this series appeared within an observation period of two years.

The majority of the group (14 patients) had gradually progressive lesions, as observed in serial films. In 3 patients the associated pleural effusion obscured the parenchymal changes (figure 1*D*).

Methods of clinical diagnosis: In addition to the roentgenographic findings, all of the ancillary clinical and laboratory techniques used in this group were evaluated for their diagnostic yield. It should be noted that complete systematic studies, i.e., bronchoscopy, scalene node biopsy, sputum cytology, and/or lung or pleural biopsy, were not performed in all patients. As

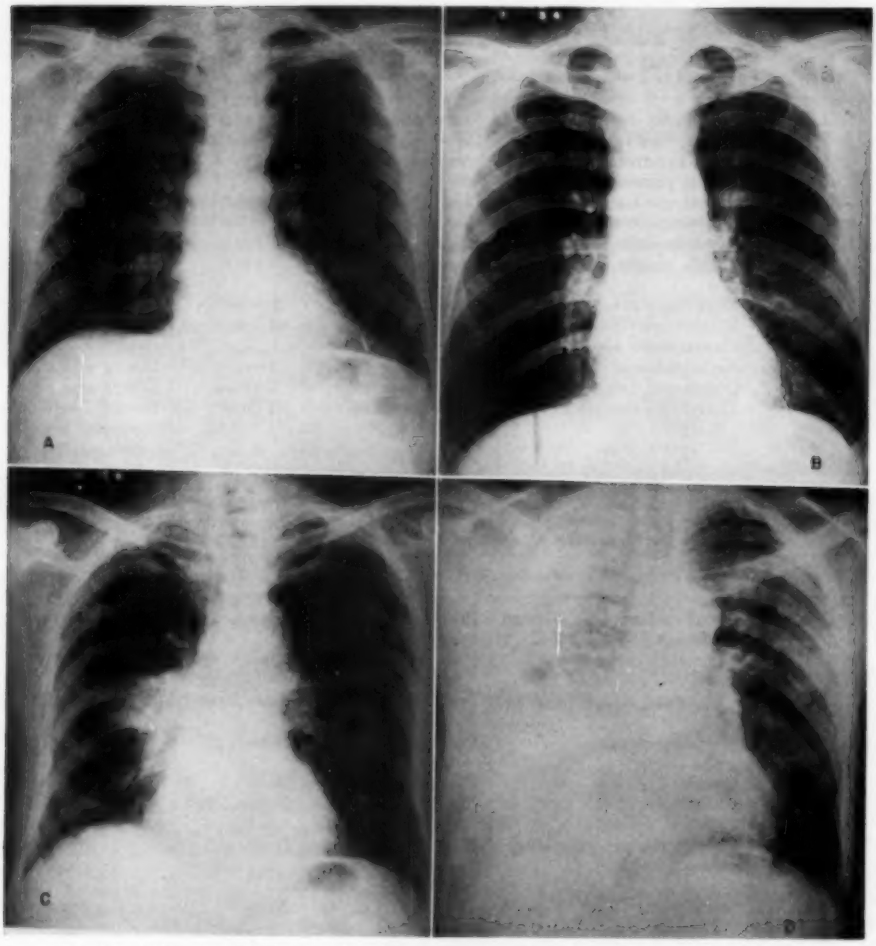

FIG. 1*A*. A 62-year-old white male with 2-cm. circumscribed peripheral right lung lesion absent on three annual films. Died two years after right upper lobectomy with cerebral metastasis.

B. A 62-year-old white male with 1.5-cm. left parahilar nodule appearing within two years' observation period. Biopsy of both axilla and supraclavicular nodes positive for metastatic adenocarcinoma. Patient died without treatment ten months after discovery film.

C. A 73-year-old white male, right superior segment density, four months' duration. Right pneumonectomy. Died on seventh postoperative day of pulmonary edema. Microscopic tumor was present in left lung.

D. A 37-year-old white male with symptoms for five months. Right pleural effusion positive for adenocarcinoma. Died one week following diagnosis. Total course, 21 weeks.

this study extends over the eight-year period, 1950–1958, several of these procedures were little used in the earlier years. Also, in patients with definite peripheral asymptomatic coin lesions without sputum production, such studies as bronchoscopy and scalene fat pad biopsy were purposely omitted.

Bronchoscopy was carried out in 9 patients. In only one was a positive biopsy obtained. In another, indirect evidence of tumor was appar-

ent. Bronchial secretions were reported negative for malignant cells in 4 patients and showed atypical cells in 4 patients. The only positive cytology from a bronchial aspirate was in the same patient with the positive bronchial biopsy. Multiple sputum studies were carried out in 10 patients more recently admitted. These were negative for malignant cells in 5 and showed atypical cells in 2 patients. In 3 patients exhibiting frankly positive cells in the sputum, these findings were usual after several preceding negative studies and in the near-terminal phase of the disease.

In the 3 patients with pleural effusion, 2 had malignant cells in the aspirate. One was associated with bloody fluid, and the cells were consistent with adenocarcinoma. In the other, there was a heavy mucinous fluid with cells consistent with mucinous alveolar cell carcinoma.

Although performed in only 5 patients, scalene node biopsy was positive for metastatic tumor in 3, including an axillary node in one patient. The histologic findings were usually interpreted as adenocarcinoma consistent with bronchiolo-alveolar carcinoma. As anticipated when the scalene nodes contained a tumor, the patient usually had evidence of fairly widespread pulmonary and extrapulmonary disease.

Pathologic Findings

The gross and microscopic structure of bronchiolar carcinoma is in most instances distinct and has been adequately described in previous publications (7–9) (figures 2, 3). Postmortem examination was performed in 17 of the nonsurviving patients. Twelve patients had evidence of extrathoracic metastasis. There was metastasis to abdominal viscera (chiefly liver and abdominal lymph nodes) in 7 cases and central nervous system metastasis in 5 cases. In 4 cases, metastasis was limited to the mediastinal and hilar lymph nodes with involvement of both pleural cavities. Lymphatic invasion of the intrapulmonic lymphatics was demonstrable in 8 cases (53 per cent). Small nests of tumor cells were seen in the peribronchial or perineural lymphatic spaces, and only rarely were the subpleural lymphatic spaces involved (figure 4). Evidence of blood vessel invasion was noted in one case. Clusters of tumor cells lying free in the alveolar spaces were noted in all cases. Dislodgment of these tumor cells could have occurred post mortem in the removal of the lungs and preparation of the sections; however, the likelihood of these tumor cells' being dislodged during life is feasible.

Proliferation of bronchiolar epithelium which occurs in certain instances of pulmonary fibrosis is not infrequently a distinct problem to distinguish from bronchiolar neoplasia. When the areas of epithelial proliferation form tumor-like nodules in relation to terminal bronchioles they often closely mimic true neoplasia both grossly and microscopically.

Surgical Treatment

Twelve patients received definitive operation in the form of six lobectomies and six pneumonectomies (*see* table 1). Lobectomy was thought to be curative in 4, although in one patient lobectomy followed an exploratory craniotomy by three years (*A.W.*). Of the 2 remaining patients who received lobectomy, one had limited pulmonary reserve and the second had a frankly palliative resection followed by postoperative X-ray therapy.

In the group receiving pneumonectomy, 5 of the 6 patients had no evidence of extrathoracic spread at the time, while the remaining patient had a so-called palliative procedure. There was one death from pulmonary edema on the seventh postoperative day.

Nonsurgical Treatment

In 9 patients, evidence of widespread metastatic disease contraindicated any attempt at definitive or palliative surgery. One patient with the diagnosis established by exploratory thoracotomy had a nonresectable lesion treated by postoperative irradiation. Two patients had pleural effusions with positive cytology, and 3 had metastatic tumors in supraclavicular nodes. In only one patient the diagnosis was not established until postmortem examination. In the 3 patients who received, respectively, systemic nitrogen mustard, intrapleural P_{32}, and a tumor dose of X-ray (7,000 r), there was no evidence that this treatment modified the ultimate course of the disease.

Results of Treatment and Current Status

In the surgically treated group the mean survival period was 27 months, with extremes of one week to nine years. As a group, those patients treated with pneumonectomy survived an average of 3.8 years, excluding the patient who

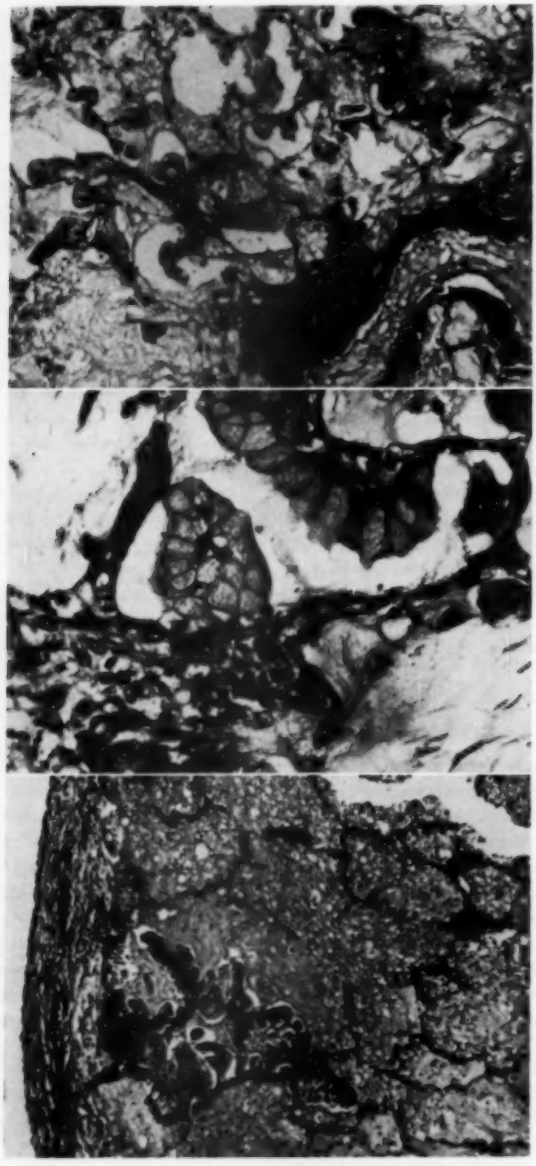

FIG. 2 (Upper). Section showing terminal bronchiole surrounded by bronchiolar carcinoma. Isolated tumor cells are seen lying in the mucous secretion of dilated alveoli. (Hematoxylin and eosin stain, × 400)

FIG. 3 (Center). Photomicrograph of bronchiolar carcinoma showing mucin-filled tumor cells attached to alveolar wall. (Hematoxylin and eosin stain, × 500)

FIG. 4 (Lower). Isolated focus of bronchiolar carcinoma beneath pleura. (Hematoxylin and eosin stain, × 100)

died in the first postoperative week. The 6 patients treated by lobectomy alone survived an average period of 15 months.

The mean survival period in the nonsurgical group of 9 patients was 11 months. However, this includes the patient still living after five years with a questionable diagnosis of bronchiolo-alveolar carcinoma. If this case is excluded, the remaining 8 patients have died, with a mean survival period of only five months. This dismal figure reflects the advanced stage of the disease when the diagnoses were established.

Of the entire group of 21 patients, 3 of those surgically treated are alive and one untreated patient is living (the latter with an equivocal diagnosis by lung biopsy). The combined survival is 18 per cent. However, only one patient treated with pneumonectomy has survived beyond five years. Of the 17 deaths, all but 2 (coronary thrombosis and postoperative pulmonary insufficiency) were the result of carcinoma.

Discussion

The concept of origin of bronchiolar carcinoma from the epithelial lining of terminal bronchioles rather than of alveoli is no longer tenable since the alveoli have been shown by Low (10) and others (11) to have a definite epithelial lining. With the use of the electron microscope, the alveolar epithelial cells or epicytes are seen to be distributed over the alveolar wall. Fine cytoplasmic extensions cover the alveolar surfaces of the capillaries. Since the attenuated portions of the alveolar epithelial lining cells are at or beyond the practical limits of resolution of the light microscope, it is only rarely that the epithelial continuity can be noted. Although definitive proof of the exact site in the bronchial tree of bronchiolar carcinoma is lacking, it is now necessary to include the alveoli as a possible site.

The subject of viruses has been of particular interest in the study of bronchiolar carcinoma. Dungal and others (12, 13) have demonstrated the presence of a virus in jaagsiekte, a primary neoplasm of the lung in sheep which closely simulates bronchiolar carcinoma in man. Dungal has observed jaagsiekte developing in healthy sheep housed with sick sheep. In 2 of the present cases (*W.F.* and *P.S.*) cultures for virus were made on fresh surgically removed tumor. Representative specimens were inoculated into tissue cultures of monkey kidney cells and subcultured at ten days with a second passage in fifteen days. No infectious agent was isolated in three passages.

Diagnostic methods commonly employed in screening carcinoma of the lung suspects would appear to be of little help in this particular tumor. It would seem a priori, since this carcinoma is characteristically a peripheral lesion often associated with the production of considerable sputum, that study of sputum cytology would furnish the highest diagnostic yield. Such studies in the patients in this report were positive only in the far advanced stage of the disease when diagnosis was largely of academic interest.

The most reliable diagnostic technique is exploratory thoracotomy. This approach has been shown to offer the greatest opportunity for prompt diagnosis and cure of indeterminate peripheral coin lesions subsequently proved to be bronchogenic carcinoma. The difficulty in applying this approach to bronchiolar carcinoma is that lobar or sublobar infiltration is commonly noncircumscribed and exudative in roentgenographic appearance, suggesting an inflammatory lesion. Consideration of thoracotomy was often delayed in the present group because the pulmonary involvement was consistent with a subacute or unresolved pneumonia or tuberculosis.

A further difficulty in early diagnostic or therapeutic attempts is the coexistence of a precocious symptomatic neural metastasis and a minimal silent pulmonary lesion. Two of the patients had thoracotomy subsequent to neurosurgical exploration but without evidence of significant palliation.

Favorable results of surgery are evident in 2 patients with minimal lesions; one is still alive four years after lobectomy and the second survived five years after pneumonectomy. Two patients with moderately advanced lesions, both treated by pneumonectomy, are alive three and nine years, respectively.

Exploratory thoracotomy and frozen section diagnosis may place both the pathologist and surgeon in a diagnostic dilemma in specific instances. The diagnosis in a typical case can be established with reasonable certainty at thoracotomy with frozen section of tissue gained by needle or wedge biopsy.

The surgeon in such instances may elect to perform a lobectomy for purposes of diagnosis and treatment, thus widely encompassing the palpable lesion or lesions. Faced with the situation of no apparent involvement in the remaining lobe and, providing pulmonary function is adequate, the surgeon must determine if pneumonectomy will enhance the prospect of cure. In two of the cases treated by pneumonectomy, ipsilateral lower lobe metastasis, possibly from aerogenous spread, was present in the surgical specimen (*see* figure 4). In one of the patients, who died on the seventh postoperative day following pneumonectomy (in which lobectomy was not technically possible), contralateral microscopic tumor was present but had not been suspected roentgenographically.

The finding of tumor cells in the alveolar spaces of the opposite lung in 3 of the present patients would indicate the likelihood of the transportation of tumor cells via the bronchial tree by ciliary action or cough reflex. The possibility that these tumor cells may implant and give rise to secondary tumors is substantiated by evidence of adherence of the tumor cells to the alveolar wall and proliferation of fibroblasts in the area of attachment (*see* figure 3). Experimental evidence of the intrabronchial spread of tumor cells has been demonstrated (14).

Recognizing the inability of the surgeon to determine the extent of involvement by palpation, it is believed that pneumonectomy will offer a more complete chance for removal of all of an ipsilateral tumor than lobectomy or segmental resection providing function of the opposite lung is satisfactory. Appreciable survival has not been reported without surgical treatment, and the early employment of adequate resection would appear to be the only available therapy.

Summary

Clinical and pathologic observations of 21 cases of bronchiolar carcinoma are described. Six patients had lobectomies and 6, pneumonectomies. Nine patients had widespread metastatic disease that contraindicated surgery. In the surgically treated group, the mean survival period was 27 months.

A survey of the roentgenographic history of this group indicates that the lesion grows to macroscopic size within a two-year period. Almost every patient had a normal chest film from six months to two years prior to detection of the abnormal shadow.

Sumario

Observaciones sobre la Historia Natural del Carcinoma Bronquíolo-Alveolar: Lo Experimentado en 21 Casos

Se describen las observaciones clínicas e histopatológicas realizadas en 21 casos de carcinoma bronquiolar. En 6 enfermos se ejecutaron lobectomías y en 6, neumonectomías. Nueve tenían metástasis generalizadas que contraindicaban la cirugía. En el grupo tratado quirúrgicamente, el período de sobrevivencia media fué de 27 meses.

Un repaso de la historia radiográfica del grupo indica que la lesión alcanzó tamaño macroscópico en término de un período de dos años. Casi todos los enfermos tuvieron radiografías torácicas negativas de seis meses a dos años antes de descubrirse la imagen anormal.

Resume

Observation sur l'histoire naturelle du carcinome bronchio-alvéolaire. Expérience de 21 cas

Les auteurs décrivent les observations cliniques et pathologiques de 21 cas de carcinomes bronchiolaires. Six malades subirent une lobectomie, et 6 subirent une pneumonectomie. Neuf malades présentaient une forme avec métastases dispersées qui contrindiquèrent toute chirurgie. Dans le groupe traité chirurgicalement, la moyenne de période de survie fut de 27 mois.

Une étude de l'histoire radiologique de ce groupe montre que la lésion se dévelopa jusqu'à une taille macroscopique en une période de deux ans. Presque chaque malade eut une radiographie de poitrine negative de six mois à deux ans avant le dépistage d'une ombre anormale.

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NONSPECIFIC RESISTANCE TO TUBERCULOSIS[1, 2]

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Introduction

The efficacy of nonliving vaccines, as well as of extracts from tubercle bacilli, in giving rise to protection against tuberculosis has been reviewed recently by Weiss (1). Two extracts have emerged as promising in that they afforded protection to laboratory animals equal to that produced by BCG. One of these, the particulate red fraction described by Youmans and associates (2, 3), although potent, is not practicable for further studies since it is difficult to produce in adequate amounts. The second, a methanol-soluble extract of tubercle bacilli, was first described by Boquet and Nègre (4–7) and has since been characterized by others (8–11). To date the mode of action of these extracts, as well as that of living tuberculosis vaccines, is not clear. The finding that treatment of experimental animals with BCG not only results in increased resistance to tuberculosis but also raises resistance to infection with gram-negative bacilli (12) and, conversely, the observation of increased resistance to tuberculosis in animals treated with endotoxins of gram-negative bacilli (13–15) have raised questions as to how extracts of tubercle bacilli bring about protection against tuberculosis.

In view of the great gap in the minimal amounts required for protection between live vaccines, on the one hand, and of killed bacteria or bacterial fractions, on the other hand, it seemed possible that the resistance evoked by the latter substances might be essentially of a "nonspecific" nature. In a first approach toward elucidation of this problem, experiments were carried out in which fractions from tubercle bacilli, gram-negative bacilli, and yeast were tested for their ability to evoke increased resistance to tuberculosis. The minimal amounts of these substances required to give comparable degrees of resistance were ascertained and were compared with each other as well as with live and heat-killed cells, and cross-protection experiments were carried out.

Materials and Methods

Tubercle bacilli fractions: One hundred grams of washed, lyophilized tubercle bacilli (H37Ra strain of *Mycobacterium tuberculosis* var. *hominis*[3]) were extracted repeatedly with methanol at 25° C. until no color was apparent in the extract. The residue was then extracted at least ten times for one-hour periods with methanol at 65° C. in a refluxing apparatus, and the pooled hot methanol extracts were Seitz filtered. Upon cooling, a white precipitate formed which was separated by centrifugation in the cold. The white precipitate was washed with cold methanol and labeled crude white. The tan clear supernatant was concentrated and dried under vacuum. The dried extract was next washed with cold methanol and labeled crude brown. Various purified subfractions of both crude extracts were obtained by chromatographic separation on a magnesium silicate-celite column as described by Bloch and co-workers (16).

Waxes C and D were extracted from washed lyophilized tubercle bacilli (strain H37Ra) according to methods outlined by Noll (17). A wax fraction originally described by Anderson (18) as a purified wax was extracted from washed tubercle bacilli (strain H37Ra).

Endotoxins: Purified *E. coli* 0127 endotoxin was obtained from a sample given by Dr. M. Landy to the late Dr. J. Freund.

Salmonella typhosa endotoxin was produced by the precipitation with acetone of an N/4 TCA extract of *S. typhosa* 0901.

Miscellaneous: Pseudomonas polysaccharide was purchased from Flint, Eaton and Co., Decatur, Illinois, and zymosan from Standard Brands Incorporated, New York, New York. Glycogen was purchased from Nutritional Biochemicals Inc., Cleveland, Ohio.

Denatured horse serum globulin was a 50 per cent ammonium sulfate precipitate of horse serum, dialyzed against saline, and heated at 60° C. for one hour.

Differences in survival time after challenge with *M. tuberculosis* var. *hominis* strain H37Rv[3] were

[1] From the Division of Applied Immunology, Public Health Research Institute of New York, New York, New York.

[2] Supported by Research Grant E-2628 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[3] Strains of *Mycobacterium tuberculosis* var. *hominis* were obtained from Dr. William Steenken, Trudeau Laboratory, Saranac Lake, New York.

determined by the method of Youmans and associates (19).

Twenty female CF_1[4] mice, 18 to 22 gm., were injected intraperitoneally with graded doses of each fraction contained in a volume of 0.2–0.4 ml. of saline or Bayol F. After a period of time (as indicated), the mice were challenged intravenously with 0.5 mg. (wet weight) of tubercle bacilli grown on the surface of modified Proskauer and Beck medium (20). This amount of tubercle bacilli killed 95 to 100 per cent of the control mice within thirty days, with a mortality peak at approximately fourteen days. Deaths were recorded daily and autopsies performed to verify the cause of death. Both per cent survival after thirty days (per cent S-30) and mean survival time were calculated and compared with those in control groups of equal number. Where applicable, results were analyzed statistically as indicated in the tables.

Differences in survival time after challenge with *Diplococcus pneumoniae* Type III, *Salmonella typhimurium* (ATCC 6994), *S. enteritidis* (ATCC 13076), and *Klebsiella pneumoniae* McKibbin were determined as follows: One hundred and twenty female CF_1 mice, 18 to 22 gm., were injected intraperitoneally with a constant dose of the tubercle bacilli fractions to be assayed. After one to four weeks, groups of 10 mice each were challenged by intraperitoneal injection of a diluted twenty-four-hour brain-heart infusion broth culture in a volume calculated to contain approximately 10 LD_{50} doses of bacteria. The LD_{50} dose of each microorganism is listed in the tables.

Results

In order to determine the efficiency of methanol extraction as well as to compare the protective activities of treated and untreated tubercle bacilli, mice were injected intraperitoneally with living (lyophilized) and treated suspensions of cells. The survival time of mice subjected to challenge with virulent tubercle bacilli (per cent S-30) after treatment with these vaccines is shown in table 1. The lyophilized vaccines were extremely potent since 0.001 mg. (wet weight) produced an S-30 of 60 per cent, while 1.0 mg. (wet weight) of extracted cell residue was required to give the same protective action. Note also that heating for thirty minutes at 100° C. of whole cells resulted in the nearly complete loss of protective activity, since 0.1 mg. (wet weight) no longer produced a protective effect in mice. Control animals, injected with saline or Bayol F never showed an S-30 greater than 15 per cent.

Since the methanol-extracted cell residue (approximately 68 per cent of the original weight) was of such low protective potency, the material in the extracts was assayed next. Twenty-six per cent of the cell material went into solution after extraction with cold methanol, but this extract was free of detectable protective activity (table 2). Hot methanol dissolved another 6 per cent of the cell material which was separated into two parts, crude white and crude brown, each of which proved to be protective. However, it appeared that much more of the crude extracts (table 2) was required for a significant S-30 than could be accounted for in the amounts of living cells producing the same S-30 (table 1). Crude brown appeared more potent than crude white, since 2.0 mg. (dry weight) of crude brown protected mice to the same degree as 20.0 mg. of crude white.

Possible explanations for the low activity of the crude extracts might be that either the protective material was destroyed by the extraction procedure or that masking substances were liberated. If the latter were true, then their removal should theoretically produce a fraction with the potency of intact cells on a net weight basis. To test for this possibility, crude extracts were fractionated on magnesium silicate-celite columns and eluted with various solvents (tables 3, 4). Of the crude white subfractions (table 3),

TABLE 1

Protective Effect of Different Numbers of Living and Treated M. Tuberculosis var. Hominis H37Ra Cells against Tuberculous Infections in Mice

Treatment	Amount per Mouse	Per Cent Survival after Thirty Days
	mg. (wet weight)	
Lyophilization	1.0*	80–100
Lyophilization	0.1	80–100
Lyophilization	0.01	80
Lyophilization	0.001	60
Lyophilization	0.0001	10–20
Residue of extraction with methanol (65°C.) (*see* text)	1.0	60
Residue of extraction with methanol (65°C.) (*see* text)	0.1	10
Heated 100°C. 1 hour	1.0	30
	0.1	10

* 1.0 mg. (wet weight) contains approximately 40,000,000 cells (2). *See* text for procedure.

[4] Purchased from Carworth Farms, New City, Rockland County, New York. Animals were quarantined for two to three weeks prior to use.

TABLE 2

COMPARATIVE ACTIVITIES OF CRUDE 65°C. METHANOL EXTRACTS OF H37Ra

Fraction	Amount per Mouse	Per Cent Survival after Thirty Days
	mg. (dry weight)	
Methanol extract, 25°C.	20.0	0
Crude white 65°C. methanol extract	20.0	25–52
Crude white 65°C. methanol extract	2.0	10–20
Crude white 65°C. methanol extract	0.2	10–20
Crude brown 65°C. methanol extract	20.0	80
Crude brown 65°C. methanol extract	2.0	45
Crude brown 65°C. methanol extract	0.2	10

All fractions were dissolved in Bayol F.

TABLE 3

ACTIVITY OF CRUDE WHITE FRACTIONS ELUTED FROM MAGNESIUM SILICATE-CELITE COLUMN

Fraction Number	Eluted with 50 Ml. of	Yield*	Minimal Protective Dose	Per Cent Survival after Thirty Days
		mg.	*mg.*	
1–6	Petroleum-ether	410	16.0	35
7	Petroleum-ether-benzene (1-1)	32	1.28	20
8	Petroleum-ether-benzene (1-1)	105	4.2	40
9	Petroleum-ether-benzene (1-1)	21	8.4	25
10	Petroleum-ether-benzene (1-1)	5	0.2	35
11	Petroleum-ether-benzene (1-1)	4	—	0
12–16	Ether—10% methanol	212	—	0
17	Benzene	14	0.56	21
18–25	Acetone, chloroform, hot methanol	50	—	0

Fractions dissolved in Bayol F.

Control mice (Bayol F only) 0–5% survival after thirty days.

* Recovery—57%.

three fractions, 8 to 10, had protective activity equal to or better than that of the crude. Fraction 10, in terms of minimal effective dose, possessed 100 times the activity of the crude starting material.

To determine whether this increase was due to the removal of masking substances, two hypothetic situations were considered. The first involved the assumption that the column was selective and that, of the total of the recovered material (57 per cent), the loss (43 per cent) reflected a retention of masking material and complete elution of protective fractions. If this were the case, then a theoretic distribution as shown in table 5 (*A*) would be expected. On the other hand, if the column was nonselective and the 43 per cent loss reflected a uniform loss of 43 per cent of all of the fractions, then a theoretic distribution as shown in table 5 (*B*) would be expected. The sum of minimal effective doses per 20.0 mg. in each case was approximately 2.0, which means that crude white contained no more than two minimally effective doses in 20.0 mg. It is therefore considered unlikely that masking substances existed, and in all probability the activity of crude white was due to the combined effect of all of the active fractions.

Only one fraction of crude brown (table 4), that eluted with hot methanol, was active in an amount equivalent to the starting material (1.12 compared with 2).

The activity of fractions purified by selective solubility in petroleum-ether and acetone are shown in table 6. Only the petroleum-ether-insoluble fractions of the crude extracts produced significant increases in survival time as well as significant S-30 values. Note, however,

TABLE 4

ACTIVITY OF CRUDE BROWN FRACTIONS ELUTED FROM MAGNESIUM SILICATE-CELITE COLUMN

Fraction Number	Eluted with 50 Ml. of	Yield*	Minimal Protective Dose	Per Cent Survival after Thirty Days
		mg.	*mg.*	
1–6	Petroleum-ether	339		0
7–11	Petroleum-ether-benzene (1-1)	5		0
12–16	Ether—10% methanol	159		0
17	Benzene	6		0
18	Acetone	8		0
19	Chloroform	1		0
20–25	Hot methanol	280	1.12	40–50

Fractions dissolved in Bayol F.

Control mice (Bayol F only) 0–5% survival after thirty days.

* Total recovery—65%.

TABLE 5

THE CALCULATED AND EXPERIMENTAL DISTRIBUTION OF ACTIVITIES OF CRUDE WHITE SUBFRACTIONS BASED ON THE HYPOTHETIC PRESENCE OR ABSENCE OF MASKING SUBSTANCE

A

Assume that the column is selective and that the 43% loss reflects retention of masking substances with complete elution of protective fraction

Fraction	Recovery	Calculated Content in 20 Mg.	M.P.D.*	Number of M.P.D per 20 Mg. of Crude White
	mg.		*mg.*	
1–6	410	5.40	16.0	0.34
7	32	0.42	1.3	0.33
8	105	1.40	4.2	0.33
9	21	0.28	8.4	0.03
10	5	0.07	0.2	0.33
11	4	0.05	—	—
12–16	212	2.80	—	—
17	14	0.18	0.6	0.3
18–25	50	0.66	—	—
Total 853 (57% recovered)				Total 1.63

B

Assume column is nonselective and that 43% loss reflects a uniform loss of all components. Then the per cent of total material recovered equals the per cent of fraction in original material

Fraction	Recovery	Per Cent of Total Recovered	M.P.D.*	Calculated Content in 20 Mg. of Crude White†	Number of M.P.D. per 20 Mg. of Crude White
	mg.			*mg.*	
1–6	410	48.0	16.0	9.6	0.6
7	32	3.0	1.3	0.5	0.3
8	105	12.0	4.2	2.4	0.57
9	21	2.0	8.4	0.4	0.05
10	5	0.5	0.2	0.1	0.5
11	4	0.5	—	0.1	—
12–16	212	24.0	—	4.8	—
17	14	1.6	0.6	0.3	0.5
18–25	50	5.8	—	1.2	—
Total 853 (57% recovered)					Total 2.52

* Minimal protective dose

† Crude white 65° C. methanol extract.

that for a significant S-30 the required amount of crude white petroleum-ether-insoluble fraction was equal to that of crude white (table 2). Furthermore, the crude brown petroleum-ether-insoluble fraction was less active than crude brown, since a minimum of 10 mg. was required for an S-30 produced by only 2 mg. of crude brown (table 2). The petroleum-ether-insoluble fraction of only crude white produced a significant increase in survival time when as little as 1 mg. was injected per mouse. However, this was the case only when the interval of time between treatment and challenge was two weeks. When the challenge was postponed to four weeks, no activity was seen. Intervals of time longer than four weeks or less than two were not tested.

Other tubercle bacilli fractions (table 7) produced no significant increases in S-30. Waxes C and D proved toxic in amounts as low as 1 mg. (not shown), causing the death of some of the animals within forty-eight hours.

In table 8 the results are shown of experiments designed to determine whether substances other than those derived from tubercle bacilli could increase survival time of mice challenged with virulent tubercle bacilli. Zymosan and lipopolysaccharides of *E. coli* and Salmonella proved highly effective, although somewhat toxic. It should be noted that 25°C. and 65°C. methanol extracts of *E. coli* produced increases in survival time in mice to challenge with virulent tubercle bacilli. The order of these increases

TABLE 6

ACTIVITY OF SEMIPURIFIED EXTRACTS OF H37Ra

Fraction	Mg. per Mouse	Time of Challenge	Per Cent Survival after Thirty Days
	dry weight	*weeks*	
Crude white, petroleum-ether insoluble	20	4	55
	10	4	0
	10	2	0*
	1	2	0†
Crude white, petroleum-ether soluble	20	4	0
	2	4	0
Crude brown, petroleum-ether soluble	20	4	10
Crude brown, petroleum-ether insoluble	10	4	60

All fractions dissolved in Bayol F.

* Significant increase in median survival time—t: 4.09 (P: <0.01).

† Significant increase in median survival time—t: 2.11 (P: <0.05).

was about the same as those produced with 65°C. methanol extracts of tubercle bacilli and Landy's lipopolysaccharide. These extracts, consisting mainly of lipopolysaccharides, were nontoxic in the amounts used. Neither glycogen nor Pseudomonas polysaccharide proved protective.

Whether crude white or crude brown could alter the host's response to challenge with other pathogenic bacteria was next determined (table 9). It may be seen that the crude fractions were capable of increasing the survival time of mice after infection with 10 LD_m doses of the pathogens tested. Crude white and crude brown appeared to be equal in activity within the limits of the assay. The experiment was terminated at the end of five days, since all animals succumbed to the infection by the seventh day. Challenging with less than 10 LD_m doses led to more prolonged survival but did not cause an over-all change in the results. In every case it appeared that two- and four-week intervals between treatment and challenge allowed a greater number of mice to survive than a one-week interval.

It has been shown that a variety of substances could bring about an increase in resistance as a result of stimulating the phagocytic cells of the reticuloendothelial system. One such substance, unrelated to methanol extracts or lipopolysaccharides, is denatured globulin (21). To determine whether the increased activity of phagocytes could also account for resistance to tuberculosis, mice were injected intravenously with 0.5 mg. of heat-denatured horse serum globulin on three successive days. Giving more than this amount per injection proved 100 per cent fatal. Controls were injected with saline. All animals were challenged with virulent tubercle bacilli twenty-four hours after the last injec-

TABLE 7

EFFECTS OF VARIOUS EXTRACTS OF H37Ra ON RESISTANCE TO TUBERCLE BACILLI

Extract*	Time of Challenge	Mg. per Mouse	Per Cent Survival after Thirty Days
	weeks	*dry weight*	
Acetone at 25°C.	4	20.0	0
Toluene at 25°C.	4	17.6	20
Wax C (Noll)	4	8.0	20† (toxic)
Wax D (Noll)	4	20.0	0† (toxic)
$CHCl_3$ extract of residue	4	48.0	0
Anderson's purified wax	4	1.0	11

Fractions dissolved in Bayol F.
* Extracts from lyophilized H37Ra.
† Toxic fractions killed 20–50% in 48 hours.

TABLE 8

EFFECT OF VARIOUS SUBSTANCES ON ABILITY TO ALTER RESISTANCE TO CHALLENGE WITH VIRULENT TUBERCLE BACILLI

Substance	Amount per Mouse	Time of Challenge	Per Cent Survival after Thirty Days	Median Survival Time Significance
	mg.			
S. typhosa 0901 N/4 TCA extract acetone PPT	0.2 (saline)	48 hours	0	17.6 ± 1.32 t: 2.17 P: <0.05
S. typhosa 0901 N/4 TCA extract acetone PPT	0.2 (saline)	30 days	0	Insignificant
S. typhosa 0901 N/4 TCA extract acetone PPT	0.2 (Bayol F)	30 days	0	Insignificant
E. coli cullen methanol extract at 25°C.	7.5 (Bayol F)	30 days	39	—
E. coli residue of above extracted with methanol at 65°C.	0.9 (Bayol F)	30 days	21	21.26 ± 2.65 t: 3.17 P: <0.01
E. coli 0127: B 8* (Landy)	0.4 (Bayol F)	30 days	65	—
Zymosan†	1.0 (saline)	30 days	45	—
Glycogen	20 (saline)	30 days	0	Insignificant
Pseudomonas polysaccharide	10	30 days	0	Insignificant

* Toxic: 3/20 killed in 48 hours.
† Toxic: 2/20 killed in 48 hours.

TABLE 9

EFFECT OF CRUDE TUBERCLE BACILLI EXTRACTS ON SURVIVAL TIME TO CHALLENGE WITH PATHOGENIC MICROORGANISMS

Time of Challenge	Dead/Total* (5 Days)							
	S. enteritidis		*S. typhimurium*		*D. pneumoniae* III		*K. pneumoniae* McKibbin	
	CW†	CB‡	CW	CB	CW	CB	CW	CB
weeks								
1	7/10	10/10	6/10	10/10	10/10	10/10	4/10	10/10
2	3/10	5/10	3/10	6/10	5/10	5/10	2/10	2/10
4	2/10	4/10	4/10	6/10	4/10	6/10	4/10	4/10

* Results at termination of experiment—5 days. All control animals were dead within 48 hours.

† Crude white—65°C. methanol soluble white extract.

‡ Crude brown—65°C. methanol soluble brown extract.

Each mouse received crude extract intraperitoneally (20.0 mg. of crude white, or 2.0 mg. of crude brown). Controls received only Bayol F. At the times indicated, all mice were challenged intraperitoneally with 10 LD_{50} doses of bacterial suspension. The LD_{50} dose for each was:

S. enteritidis, 2.5×10^4
S. typhimurium, 10^7
D. pneumoniae III, 10^1
K. pneumoniae, 9×10^3

TABLE 10

EFFECT OF REPEATED INTRAVENOUS INJECTIONS OF HEAT-DENATURED HORSE GLOBULIN ON RESISTANCE TO CHALLENGE WITH VIRULENT TUBERCLE BACILLI (H37Rv)

	Number of Mice	Median Survival Time	Difference
Control	19	18.73 ± 1.9	Insignificant
Treated	18	20.72 ± 3.94	t: 1.88 P: >0.05

Treated mice injected intravenously with 0.2 ml. (0.5 mg.) of heat-denatured horse globulin on 3 successive days. Controls received equal volumes of saline. All animals were challenged 24 hours later with 0.5 mg. of H37Rv.

tion (at which time the effect by the denatured protein was optimal (21)). Results (table 10) showed that the treatment did not result in increased resistance to tuberculosis.

DISCUSSION

To equal the protective activity of living tubercle bacilli, the minimal protective dose of extracts or subfractions would have had to approach that of the minimal effective dose of whole cells. Since this was not the case, it might be postulated that either a major portion of the cell's protective activity was destroyed by the extraction procedure or that masking substances were liberated. The latter was found to be unlikely, however, in view of the results obtained with chromatographically separated components of crude fractions.

The results with fractions purified by selective solubility in petroleum ether and acetone differed from those reported by Williams and Dubos (11). They reported that their F1 fraction, equivalent to a sub-fraction of crude white (the petroleum-ether-insoluble fraction) was more active than other fractions and merited further study. Tested in the manner described here, no significant increased survival after thirty days was found even in concentrations equivalent to those used with the crude extracts. However, their finding that the F1 fraction produced a statistically significant increase in survival time in those animals challenged with tubercle bacilli two weeks after administering the fraction was confirmed. Only one of their fractions, the FIII, produced a significant increase in thirty-day survival (50 per cent) and only in a concentration of 10 mg. per mouse. The possible explanation for these discrepancies might be the difference in assay procedures.

From the analogous results with zymosan and other lipopolysaccharides, it is tempting to assume that the increased resistance produced in mice by injecting methanol extracts was nonspecific. It is interesting that the methanol extract of *E. coli* was also protective and not toxic, while lipopolysaccharides from the same species proved protective and somewhat toxic. Methanol extraction may possibly cause the release of protective substances from a loose association with other substances that are toxic. The substance in methanol extracts may be analogous to Westphal's (22, 23) "lipid A" fraction, which was shown to be nontoxic while still possessing the nonspecific protective activity of the lipopolysaccharide from which it was derived. The activity of methanol extracts in mice challenged

with other pathogenic bacteria lends weight to the hypothesis of their nonspecificity.

The mechanism by which animals become resistant to infections after receiving a variety of unrelated substances is not clear. Halpern (21) reported that repeated intravenous injections of denatured globulin caused an increase in resistance to the injection of various gram-negative bacteria. This could also be correlated with an increased phagocytic activity. Others (12) have reported that animals infected with tubercle bacilli, strains BCG and H37Rv, became more resistant to challenge with gram-negative bacteria. This too could be correlated with an increased phagocytic activity. However, repeated injections of denatured horse globulin failed to produce an observable altered response in mice to challenge with tubercle bacilli. The reason for this discrepancy remains obscure.

Summary

In confirmation of the reports of others (1), it was found that methanol extracts of tubercle bacilli were capable of increasing the survival time of mice challenged with virulent tubercle bacilli. However, these extracts did not account for the total activity of lyophilized (living) suspensions. The tremendous difference in the protective activity of the lyophilized suspensions as compared with that of the extracts suggested that some substance(s) present in lyophilized cells were labile to methanol treatment. An alternate possibility (masking substances to account for the low protective activity of extracts) was excluded by an analysis of the quantitative distribution of fractions after chromatography of the crude extracts.

Waxes C and D and Anderson's purified wax were ineffective in protecting mice against tuberculosis, while lipopolysaccharides from *E. coli*, Salmonella, and a methanol extract of *E. coli* proved as effective as methanol extracts and subfractions of tubercle bacilli.

Mice treated with methanol extracts were able to resist, to a certain degree, infection by *K. pneumoniae*, *D. pneumoniae*, and two strains of Salmonella.

Sumario

Resistencia Anespecífica a la Tuberculosis

Confirmando lo comunicado por otros autores (1), se observó que los extractos metanólicos de los bacilos tuberculosos poseían la capacidad de acrecentar el tiempo de sobrevivencia de los ratones provocados con bacilos tuberculosos virulentos. Sin embargo, estos extractos no explican la actividad total de las suspensiones liofilizadas (vivientes). La tremenda diferencia en la actividad protectora de las suspensiones liofilizadas, comparadas con los extractos, sugirió que alguna(s) sustancia(s) presente(s) en las células liofilizadas era(n) lábil(es) al tratamiento con metanol. Una posibilidad alternativa (la intervención de sustancias enmascaradoras que explicaran la poca actividad protectora de los extractos) quedó excluída por un análisis de la distribución cuantitativa de la fracciones después de la cromatografía de los extractos crudos.

Las ceras C y D y la cera purificada de Anderson se mostraron ineficaces para proteger a los ratones contra la tuberculosis, mientras que los lipopolisacáridos de la *E. coli* y de Salmonellas y un extracto metanólico de la *E. coli* resultaron tan eficaces como los extractos metanólicos y las subfracciones de bacilos tuberculosos.

Los ratones tratados con extractos metanólicos se mostraron capaces de resistir, hasta cierto punto, la infección por *K. pneumoniae*, *D. pneumoniae* y dos cepas de Salmonella.

Resume

Résistance non-spécifique à la tuberculose

Pour confirmer les rapports d'autres personnes (1), on a trouvé que les extraits au méthanol des bacilles tuberculeux étaient capables d'accroitre la durée de survie de souris attaquées par des bacilles tuberculeux virulents. Cependant, ces extraits ne furent pas responsables de l'activité totale des suspensions (vivantes) lyophilisées. L'énorme différence entre l'activité protectrice des suspensions lyophilisées, comparée à celle des extraits, fait penser que certaines substance(s) présentes dans les cellules lyophilisées, sont volatilisées par le traitement au méthanol. Une autre possibilité (masquant les substances responsables de l'activité faiblement protectrice des extraits) a été éliminée par l'analyse de la distribution quantitative des produits aprés chromatographie des extraits bruts.

Les cires C et D et la cire d'Anderson purifiée furent inefficaces pour protéger les souris contre la tuberculose, tandis que les lipopolysaccharides de *E. coli*, Salmonella et un extrait au méthanol de *E. coli*, se montrèrent aussi efficaces que des extraits au méthanol et des sous-produits de bacilles tuberculeux.

Des souris, traitées avec des extraits au méthanol, purent résister, à un certain degré, à l'infec-

tion par *K. pneumoniae*, *D. pneumoniae*, et deux souches de Salmonella.

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THE INTERRELATIONSHIPS OF PRESSURE, FLOW, AND VOLUME DURING VARIOUS RESPIRATORY MANEUVERS IN NORMAL AND EMPHYSEMATOUS SUBECTS[1,2]

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Introduction

Ventilatory mechanics are usually quantified in terms of pressure-flow, pressure-volume, or volume-time relationships. Recently, the importance of the flow-volume relationship has been recognized (1). It follows that the mechanics of ventilation could be characterized rather completely by recording the simultaneous interrelationships of pressure, flow, and volume. Heretofore there has been no systematic attempt to analyze these three variables simultaneously.

This report presents a method which permits the interrelationships between transpulmonary pressure, air flow, and volume, i.e., lung inflation, to be examined simultaneously. The method consists of plotting flow against volume, pressure against flow, and pressure against volume separately but simultaneously on three oscilloscopes. Any respiratory maneuver then appears as a path or loop on each of the respective planes, i.e., the flow-volume, pressure-flow, and pressure-volume planes. By comparing any two of the plots, the complex interrelationships among the three variables may be studied. This technique has been applied to a group of normal and emphysematous subjects. Certain clinical and physiologic phenomena particularly well visualized by this integrated approach to ventilatory mechanics are discussed. In addition, the simultaneous values of the three variables during certain important respiratory maneuvers have been established in these subjects. Such data are not available in the literature.

Methods

Transpulmonary pressure, the difference between intra-esophageal and lateral mouth pressure, was measured with the system described by Schilder and co-workers (2). Respiratory air flow was recorded with a concentric cylinder flowmeter and appropriate sensing element (3). Volume was recorded by measuring the displacement of a Krogh spirometer with a rotational transducer.[3] In addition to writing these variables on a multichannel recorder,[4] any two of the variables could be recorded simultaneously on the X and Y axes of an oscilloscope.

All subjects were studied in the sitting position. The elasticity of the lung was evaluated by two methods. The effective compliance of the lung during quiet breathing was measured by the method of von Neergaard and Wirz (4), the results being listed as compliance in table 1. The interrupted method (5) was used to measure the static transpulmonary pressure at various degrees of lung inflation. Special attention was paid to obtaining this measurement at resting end-expiration and at the maximal inspiratory point. These values are listed in table 1 as static transpulmonary pressure.

Airflow resistance was measured during quiet breathing by choosing isovolume pressure and flow points (6).

Following the above determinations, the subject performed a series of breaths of vital capacity volume, during which maximal effort was exerted throughout inspiration and expiration. The subject was asked to empty his lungs completely and then fill them as rapidly and completely as possible. He then immediately exhaled as hard and fast and completely as possible. This sequence was repeated several times for each determination. Such a maneuver has been defined elsewhere (7) as a maximal *effort* vital capacity breath. Next, the subject's tidal breathing was recorded followed by a brief series of breaths of the type employed in the maximal breathing capacity test. The volume reference for these various maneuvers was the maximal inspiratory point. During each of these maneuvers, plots of pressure versus flow, pressure versus volume, and flow versus volume were photographed from oscilloscopes.

In all subjects a maximal *flow* vital capacity breath was also recorded (7). This is obtained by having the subject first perform a maximally rapid inspiration of vital capacity volume followed by a series of expiratory vital capacity breaths of

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[3] Microsyn Type 1C-020J, Doelcam Corporation, Boston, Massachusetts.

[4] Sanborn Model 67-1200, Sanborn Company, Cambridge, Massachusetts.

TABLE 1

PHYSIOLOGIC DATA OF SUBJECTS

Measurement	Normal		Emphysema	
	Mean	Standard Deviation	Mean	Standard Deviation
1. Vital Capacity (L)	4.6	0.5	3.1	0.7
2. Compliance (L/cm. H_2O)	0.242	0.1	0.207	0.07
3. Resistance (cm. H_2O/L/sec.)	1.98	0.8	9.41	6.5
4. MMF (L/sec.)	3.2	1.2	0.6	0.3
5. Static transpulmonary pressure (cm. H_2O)				
A. End-expiratory	−5.0	1.2	−3.3	1.6
B. MIP	−30.5	7.5	−13.0	3.8
6. Maximal expiratory flows (L/sec.)				
A. Maximal flow VC				
1. 75% VC	7.4	1.6	1.5	0.7
2. 50% VC	4.8	1.8	0.9	0.4
3. 25% VC	2.1	1.0	0.4	0.2
B. Maximal effort VC				
1. Peak flow	8.5	1.6	2.5	1.2
2. 75% VC	6.6	1.8	1.1	0.6
3. 50% VC	3.9	1.4	0.6	0.3
4. 25% VC	1.5	0.6	0.3	0.1
C. MBC	6.1	1.8	2.0	0.8
7. Maximal inspiratory flows (L/sec.)				
A. Maximal effort and maximal flow VC				
1. Peak flow	8.5	1.3	3.6	1.4
2. 75% VC	6.8	1.5	2.8	1.2
3. 50% VC	8.0	1.3	3.3	1.3
4. 25% VC	7.1	1.1	2.9	1.4
B. MBC)	6.5	1.5	2.4	1.1
8. Maximal expiratory pressures (cm. H_2O)				
A. Maximal effort VC				
1. Peak pressure	98	35	90	17
2. 75% VC	68	29.6	62	33
3. 50% VC	90	41	66	28
4. 25% VC	70	31	54	32
B. MBC	48	40	37	27
9. Maximal inspiratory pressures (cm. H_2O)				
A. Maximal effort VC				
1. Peak pressure	−35	10	−36	15
2. 75% VC	−30	9	−26	7
3. 50% VC	−29	9	−31	12
4. 25% VC	−26	9	−35	22
B. MBC	−26	9.7	−22	6

MMF = maximal midexpiratory flow
MIP = maximal inspiratory point
VC = vital capacity
MBC = maximal breathing capacity

varying effort. By plotting flow against volume it is possible to record the maximal inspiratory and expiratory flows that can be achieved at every degree of lung inflation. Additional aspects of this maneuver are discussed elsewhere (1, 7). By maintaining constant calibration factors in a given subject, it was possible to superimpose the figures obtained from the oscilloscope to provide composite graphs of the various maneuvers (figure 1).

It should be noted that the expiratory phase of the maximal effort vital capacity maneuver corresponds to a forced expiratory vital capacity breath. The inspiratory phase may be thought of as a forced inspiratory vital capacity breath.

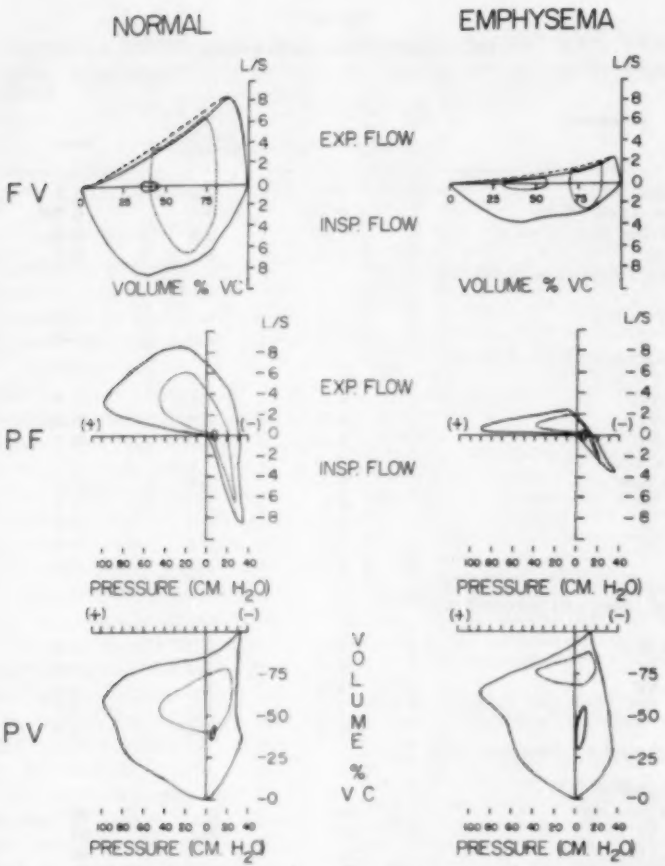

FIG. 1. Composite plots for normal and emphysematous groups. Composite flow-volume (FV), pressure-flow (PF), and pressure-volume (PV) plots for the normal and emphysematous groups. Volume is plotted as percentage of the observed vital capacity. Flow is given in liters per second. The solid line which forms the perimeter of all plots, except the expiratory portion of the FV plot, defines the maximal effort vital capacity (VC) maneuver. Its expiratory segment corresponds to a forced expiratory VC breath. The dashed lines on the expiratory portion of the FV plots depict the course of the maximal flow VC maneuver. The maximal breathing capacity (MBC) breathing is shown by the dotted loops. The small solid loops define resting tidal breathing. In the PV graph the maximal effort VC curve proceeds in a counterclockwise direction. The maximal flow VC maneuver is depicted on only the FV graph.

The inspiratory phases of the maximal effort vital capacity and maximal flow vital capacity maneuvers are identical.

The notations of maximal effort vital capacity, maximal flow vital capacity, forced expiratory vital capacity, and forced inspiratory vital capacity refer to the specific respiratory maneuvers defined above. In this report these terms will also denote the graphic representations in figure 1 of the flow-volume, pressure-flow, and pressure-volume relationships during these maneuvers.

The maximal midexpiratory flow during a forced expiratory vital capacity breath was also measured (8). All volumes were expressed at ambient temperature and pressure saturated with water vapor (ATPS).

The subjects, all of whom were males, were divided into two groups. There were 12 subjects

judged to have no significant complaints referable to the cardiopulmonary system. Mild chronic bronchitis was suspected in 4 subjects. Eight subjects were soft-coal miners and 5 of these had roentgenographic evidence of mild pneumoconiosis. The mean age was 48.6 years.

Fourteen subjects were considered to have significant degrees of pulmonary emphysema. The diagnosis of emphysema was based on the history, physical examination, routine pulmonary function studies (forced expiratory vital capacity and maximal breathing capacity), and objective evidence of restricted exercise tolerance. In all cases this diagnosis seemed consistent with the ventilatory mechanics data. All subjects in this group were miners and 5 had mild pneumoconiosis. The mean age was 56.5 years.

Results

Composite traces of the various maneuvers for both groups of subjects are presented in figure 1. These graphs were constructed from mean values for each group. Some of the values and standard deviations pertaining to figure 1 are listed in table 1. Values for maximal expiratory flow during the maximal flow vital capacity are given at 75, 50, and 25 per cent of the vital capacity (6A1-3). Values for maximal flows and pressures during the maximal effort vital capacity are given for the same volume points.

Much of the data in table 1 can be read directly from these traces. From the flow-volume plot the peak expiratory and inspiratory flows may be obtained during the maximal effort vital capacity, maximal flow vital capacity (6B1 and 7A1), and the maximal breathing capacity (6C and 7B). The flow at any volume during each of these maneuvers is also readily obtained (6A1-3, 6B2-4, 7A2-4). The pressure-flow plot also yields the peak flow values, as well as the peak expiratory and inspiratory pressures, during the maximal effort vital capacity (8A1 and 9A1) and maximal breathing capacity (8B and 9B). The static transpulmonary pressures at resting end-expiration and the maximal inspiratory point may be obtained from this plot (5A and 5B). The pressure-volume graph gives the peak expiratory and inspiratory pressures during the maximal effort vital capacity and maximal breathing capacity, the pressure at any volume during these maneuvers (8A2-4 and 9A2-4), and the static transpulmonary pressures at end-expiration and the maximal inspiratory point.

The emphysematous subjects in the study had smaller vital capacities than the normal subjects, but the effective compliance of the two groups was not significantly different (table 1). Striking differences did exist between the groups in terms of flow resistance, the maximal midexpiratory flow, and static transpulmonary pressure at the maximal inspiratory point. The static pressures at resting end-expiration showed little difference. In general, the subjects showed the expected inverse relationship between flow resistance and maximal midexpiratory flow. However, there were two individuals who showed definite reduction in maximal midexpiratory flow despite normal resistance. These subjects had values of 1.7 and 1.0 liters per second and corresponding resistances of 1.7 and 2.1 cm. of H_2O per liter per second.

In terms of pressure, flow, and volume,[8] the most striking difference between the two groups may be seen in the maximal flows developed during certain maneuvers, as indicated in figure 1. The normal subjects developed peak expiratory flows of 8.5 and 6.1 liters per second during the maximal effort vital capacity and maximal breathing capacity, respectively. The emphysematous subjects had corresponding flows of 2.5 and 2.0 liters per second. The flow-volume plots show that this degree of separation persisted throughout the entire expiratory flow-volume courses of these maneuvers.

The volume courses of maximal inspiratory flow for the two groups do not show as marked a separation. Moreover, in emphysema there is a greater reduction in maximal expiratory flow than in maximal inspiratory flow during the maximal effort and maximal flow vital capacity maneuvers. This is best seen at the mid-vital capacity, i.e., 50 per cent of the vital capacity. At this volume in the normal subjects the ratio of maximal expiratory to maximal inspiratory flow during the maximal effort vital capacity was 0.49. This ratio was reduced to 0.18 in the emphysematous group. Comparison of peak flow values (table 1) does not reveal this disproportionate reduction in expiratory flow as strikingly.

From the pressure-flow and pressure-volume plots it is apparent that both groups developed approximately the same transpulmonary pressures during the maximal effort vital capacity

[8] In the study of some respiratory maneuvers, it would be preferable to plot volume as per cent observed or predicted total lung capacity rather than as per cent of observed vital capacity.

and maximal breathing capacity. The volume courses of pressure (pressure-volume graph) were very similar for both groups during the maximal effort vital capacity. Marked variability in absolute pressures among subjects in both groups did occur, however, as evidenced by the large standard deviations (8, 9; table 1).

The volume course of maximal expiratory flow (flow-volume plot) differed from that of maximal inspiratory flow in both groups. During the expiratory phase of the maximal effort vital capacity breath, once peak flow had been attained, a semi-exponential fall in flow occurred with decreasing lung inflation. On the other hand, during the inspiratory phase, flow reached maximal values after inspiration of approximately 35 per cent of the vital capacity, and tended to stay near this level until about 80 per cent of the vital capacity had been inspired. The curve representing the maximal flow vital capacity (dashed line) followed the same course as that of the maximal effort vital capacity throughout inspiration and during early expiration until peak expiratory flow had been attained. Past this point, the maximal flow vital capacity exceeded the maximal effort vital capacity. As is also seen from the flow-volume plots, the expiratory portion of the maximal breathing capacity curves tended to lie between the maximal effort and maximal flow vital capacity curves.

An interesting relationship was seen in the flow-volume plots between the expiratory portion of the tidal breathing curve, on the one hand, and the corresponding segments of the maximal effort and maximal flow vital capacity curves, on the other. In normal subjects, the tidal breathing curve was widely separated from the other traces. In emphysematous subjects, the tidal breath almost coincided with the maximal effort vital capacity curve. In several patients the tidal breath exceeded the maximal effort vital capacity and, in 2 individuals, much of the tidal breathing curve followed the same course as the maximal flow vital capacity.

Discussion

The study of ventilatory mechanics from simultaneously obtained determinations of flow-volume, pressure-flow, and pressure-volume plots has been rewarding. Relating the three variables during various breathing maneuvers allows the characterization of most aspects of the mechanics of breathing. By combining any two sets of plotted data (figure 1), any breathing maneuver may be quantified in terms of all three variables. For example, the interrelationships of pressure, flow, and volume can be determined throughout the entire forced expiratory vital capacity maneuver by relating the flow-volume plot to the pressure-flow graph in terms of corresponding flow values. Thus, in normal subjects, the peak expiratory flow of 8.5 liters per second occurs at 88 per cent of the vital capacity and at a transpulmonary pressure of +23 cm. of H_2O.

Certain plots are particularly valuable in studying specific aspects of ventilatory mechanics. The relationship between tidal breathing and maximal expiratory flow is best visualized from the flow-volume plots which demonstrate in dramatic fashion the limited expiratory flow reserve available to the emphysematous subject. Since the maximal flow vital capacity curve defines the highest expiratory flow that can be obtained at any lung inflation, the emphysematous subject, unlike the normal subject, must in part meet any significant demand for increased ventilation by breathing at a greater degree of lung inflation. Indeed, it has been suggested that the relationship between tidal breathing and the maximal flow vital capacity as obtained from flow-volume plots might serve as an index of severity and disability in emphysema (9).

Several factors that influence the volume ventilated during the maximal breathing capacity test have been studied in detail. These include the rate of breathing, maximal expiratory and inspiratory flow rates (10), and the magnitude of transpulmonary pressure swings (11). However, there has been no evaluation of this test by considering the pressure, flow, and volume variables simultaneously. It is evident (figure 1 and table 1) that normal and emphysematous subjects develop similar pressure swings during this maneuver; hence, an inability to develop normal pressure does not explain the reduced maximal breathing capacity characteristic of emphysema. Although the emphysematous subject tended to perform the maximal breathing capacity test at near-maximal lung inflation, considerable variation was noted in the degree of lung inflation at which subjects in each group performed the test. This fact suggests that prediction of the maximal breathing capacity from some fixed segment of the forced expiratory vital capacity maneuver is of limited value. It is also apparent that the degree of lung inflation at

which the test is performed may be a major determinant of the volume ventilated, a consideration generally neglected in previous studies. The flow-volume plot of the emphysematous group may be considered as an example. If the volume per breath of the maximal breathing capacity test is held constant, performing the test at a lesser degree of inflation would reduce the volume ventilated due to the reduced expiratory flow available. Conversely, limitation of maximal inspiratory flow would reduce the volume ventilated if the test were performed at a greater lung inflation. In a given individual, knowing the flow-volume plot of the maximal flow vital capacity, the volume of the maximal breathing capacity breath, and the degree of lung inflation at which the test is performed, the greatest volume that can be ventilated and the rate that must be utilized can be calculated. Thus, the important ventilatory determinants of the maximal breathing capacity test may be studied rather simply and completely from the flow-volume plot. It should be stressed, however, that the physiologic factors determining the rate of breathing, the breath volume, and the degree of lung inflation at which a subject performs the test have not been elucidated.

The relations of volume to flow (flow-volume plot) and to pressure (pressure-volume plot) during a forced expiratory vital capacity breath indicate that the level of expiratory flow during this maneuver is not directly related to the force applied to the lung. The emphysematous group, for example, attained maximal expiratory flow at a transpulmonary pressure of only 8.4 cm. of H_2O. The experimental design of this study did not permit an absolute measurement of the pressures associated with the expiratory phase of the maximal flow vital capacity maneuver. However, preliminary data from related investigations indicate that over the lower 60 per cent of the vital capacity the transpulmonary pressures associated with maximal expiratory flow (maximal flow vital capacity) were approximately only one-fourth of those developed during the forced expiratory vital capacity. Therefore, during this latter maneuver all subjects easily developed pressures in excess of those necessary to produce truly maximal expiratory flow over this volume range. Since the ordinate values of flow for the maximal effort and maximal flow vital capacity are not too different, the forced expiratory vital capacity test should be quite reproducible over this general volume range. This agrees with reported clinical experience (8).

The subjects who developed the greatest inspiratory pressure during a forced inspiratory vital capacity test did not necessarily achieve the highest flow. However, in a given subject at a given lung inflation, the magnitude of inspiratory flow was directly related to the force applied to the lung. This finding had been predicted on the basis of the relative linearity of the pressure-flow relationships during inspiration (7).

In emphysema, attention has been focused primarily on the reduction in maximal expiratory flow characteristic of this condition. However, few data are available on the volume course of this retarded expiratory flow. It is evident from figure 1 and table 1 that comparison of maximal expiratory flows at the mid-vital capacity provides a better separation between normal and emphysematous subjects than does comparison of peak flow values. Thus, during a forced expiratory vital capacity, peak expiratory flow in the patients was 29 per cent of normal, while at 50 per cent of the vital capacity it was only 15 per cent of normal.

It has been known that maximal inspiratory flow is also reduced in emphysema. However, the volume course of this reduced inspiratory flow has not previously been measured. Furthermore, the disproportionate reduction in expiratory flow in emphysema has received little attention. McNeill and associates (12) did compare peak expiratory and inspiratory flows from volume-time curves. As in the present study, they found expiratory flow to be disproportionately reduced in emphysema. However, as noted above and as is evident from the flow-volume plots, the excessive expiratory flow reduction in emphysema is better studied at the mid-vital capacity. The implications of this predominant reduction in expiratory flow in emphysema are apparent from the emphysema flow-volume plot. To increase ventilation significantly above resting levels, these subjects must not only breathe at higher levels of lung inflation but also must make full use of the greater inspiratory flow reserve that is available. In general, the patient must achieve high flows very quickly during inspiration since expiration will proceed at lower flows and consequently occupy more time.

Normal and emphysematous subjects showed little difference in effective lung compliance. A significant reduction in lung elasticity in this

disease is revealed, however, when static transpulmonary pressures at maximal lung inflation (maximal inspiratory point) are compared. This important feature of emphysema has received only limited attention (6, 13).

Reductions in maximal expiratory flow are generally attributed to an increase in flow resistance and a decrease in lung elasticity, defects which permit marked narrowing of the bronchial tree at relatively low flow velocities and transpulmonary pressures. The majority of the present subjects fit this concept, but two interesting exceptions were encountered who had decreased maximal midexpiratory flow but normal resistance. The static transpulmonary pressure at the maximal inspiratory point was reduced in one but normal in the other. Thus, it would appear that factors other than elevated flow resistance and reduced lung retractive force were responsible for their diminished expiratory flows. The measurement of such factors has not yet been achieved (13–15). However, the fact remains that a reduction in maximal expiratory flow is not necessarily associated with an increase in flow resistance. This is an important consideration in any attempt to select a single test to detect early cases of emphysema.

The presence of pneumoconiosis in many of the subjects might conceivably have modified the mechanical behavior of the lung. This investigation does not permit any conclusions in this regard. However, a recent study of this problem revealed no correlation between compliance or flow resistance and roentgenographic severity of pneumoconiosis (16).

Summary

A method of studying the simultaneous interrelationships of pressure, flow, and lung inflation is presented. Twelve normal and 14 emphysematous subjects were studied by this integrated approach to ventilatory mechanics.

Values for pressure, flow, and lung inflation during certain clinically important respiratory maneuvers are presented. Such data provide a physiologic basis for the evaluation of many tests of ventilatory function. This is particularly true for tests quantifying maximal flow during forced expiration and for the maximal breathing capacity test.

Certain important physiologic phenomena which have received little attention in the past are particularly well visualized by this technique. For example, in emphysema, flow-volume plots show that maximal expiratory flow is reduced out of proportion to maximal inspiratory flow. Furthermore, the patient with severe emphysema at rest breathes near or at his maximal achievable expiratory flow levels. The effects of these factors on the ventilatory patterns of the emphysematous subject are discussed.

Sumario

Las Interrelaciones de la Presión, la Corriente y el Volumen durante Varias Maniobras Respiratorias en Sujetos Normales y Enfisematosos

Se presenta un método para estudiar las interrelaciones simultáneas de la presión, la corriente, y la inflación pulmonar. Con este aborde integrado a la mecánica ventiladora se estudió a 12 sujetos normales y a 14 enfisematosos.

Se presentan los valores para la presión, la corriente y la inflación pulmonar durante ciertas maniobras respiratorias clínicamente importantes. Eso datos ofrecen una base fisiológica para la justipreciación de muchas pruebas de la función ventiladora. Esto reza en particular con las pruebas que acuantian la corriente máxima durante la espiración forzada y para la prueba de la capacidad respiratoria máxima.

Ciertos importantes fenómenos fisiológicos que han recibido poca atención en el pasado se visualizan en particular bien con esta técnica. Por ejemplo, en el enfisema, las gráficas de volumen-corriente muestran que la corriente espiratoria máxima se reduce fuera de toda proporción con la corriente aspiratoria máxima. Además, el enfermo que padece de enfisema grave en reposo respira casi a, o hasta, los niveles máximos obtenibles de corriente espiratoria. Se discuten los efectos de estos factores sobre los patrones ventiladores del sujeto enfisematoso.

Resume

Les corrélations de pression, débit et volume pendant les différentes manœuvres respiratoires chez des sujets normaux et des emphysémateux

Les auteurs présentent une méthode d'étude des corrélations simultanées de pression, débit et remplissage du poumon. Il étudia douze sujets normaux et 14 emphysémateux, grace à ce moyen d'approche complet de la mécanique de ventilation.

L'auteur présente des valeurs de pression, de débit, de remplissage du poumon pendant certaines manoeuvres respiratoires importantes en clinique. De telles données offrent une base physiologique pour l'evaluation de nombreux examens de la fonction de ventilation. Ceci est particuliérement vrai pour les examens renseignent sur le débit maximum en expiration forcée et pour l'examen de la capacité maxima de respiration.

Cette technique met particuliérement bien en évidence certains phénomènes physiologiques importants qui ont reçu peu d'attention dans le passé. Dans l'emphysème, par exemple, des tracés débit-volume montrèrent que le débit expiratoire maximum est reduit sans aucune relation avec le débit maximum inspiratoire. En outre, le malade avec un emphysème sévère, respire, au repos, à des valeurs maxima—ou presque—de son débit expiratoire. L'auteur discute les effets de ces facteurs sur les modes de ventilation du sujet emphysèmateux.

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Addendum

Since the preparation of this manuscript, the following studies which utilize the same methods as those in the present report have come to the writer's attention: (*1*) Woldring, S.: The mechanics of breathing: General principles and technique of measurement, Proc Tuberc Res Council (Royal Netherlands Tuberc Ass), 1959, No. 46, 5; (*2*) Burger, H. C.: The significance of the flow/volume diagram in the study of the mechanics of breathing, Proc Tuberc Res Council (Royal Netherlands Tuberc Ass), 1959, No. 46, 28.

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RESPIRATORY SYMPTOMS AND VENTILATORY FUNCTION IN 172 ADULTS EMPLOYED IN A BANK[1]

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Introduction

The prevalence of chronic nonspecific lung disease (bronchitis, emphysema, and asthma) has not been adequately determined in the United States. Evidence suggests that these conditions are relatively common (1) and that in recent years they have taken an increasing toll in the form of disability as well as deaths (2, 3).

British investigators have pioneered work in the field of surveys to determine the frequency of symptoms and ventilatory impairment in various groups, particularly in relation to age and sex, occupation, geographic location, and smoking habits (4–9). Studies of this type are virtually unknown in this country (9, 10).

The present study was carried out in an attempt to learn something about the frequency of respiratory symptoms and ventilatory impairment in a group of adults in a sedentary, clean occupation.

Methods

All employees more than forty years old in a Denver, Colorado, bank were asked to participate in "a survey for bronchitis and asthma-like conditions." The study was limited to persons older than forty years because it is in this group that chronic nonspecific lung disease has its greatest frequency. Ninety-five (77 per cent) of 122 men and 77 (80 per cent) of 96 women volunteered and were studied. Their mean ages were fifty-five and forty-nine years, respectively.

Each subject first answered a questionnaire on respiratory symptoms consisting of the following questions:

1. Do you ever have a cough?
   a. When?
      1) More or less daily
      2) Mainly with chest colds
      3) At other times
   b. Is it a cigarette cough?
   c. How long have you coughed?
2. Do you ever cough up phlegm or mucus?
   a) When?
      1. Only with chest colds
      2. Mainly in winter with colds
      3. Even in absence of colds
      4. In the morning
   b) What color is the phlegm?
      1. Clear
      2. Yellow or green
   c) Do your colds ever go down into the chest?
3. Do you ever wheeze in your chest?
   a) When?
      1. With chest colds
      2. On exertion
      3. Other times
4. Have you had asthma?
5. Have you had pleurisy?
6. Have you had pneumonia?
7. Have you had tuberculosis?
8. Have you had sinus trouble?
9. Have you had chest colds during each of the last two winters?
10. Have you had high blood pressure or heart disease?
11. Has anyone in your family had asthma or bronchitis?
12. Can you walk and hurry as well as others your age and sex?
13. Can you keep up with others only on level walking?
14. Can you walk a mile slowly but not with others?
15. Are you short of breath after walking three blocks or less?
16. How long have you noted this change?
17. Have you ever changed jobs or moved because of cough or breathing trouble?
18. Have you ever smoked?
    a) Do you smoke now?
    b) Type (cigarette, cigar, or pipe)
    c) Amount daily
    d) Age started _____ ; age stopped _____
19. Have you seen a doctor in the past year because of breathing trouble, cough, wheezing or chest pain?

After a brief instruction period, the subject performed several forced, complete expirations into a plastic mouthpiece fitted into a large-bore rubber tube connected to a Stead-Wells spirometer, the drum of which rotated at a speed of 32 mm. per sec. Approximately ten minutes were required for questioning and testing each subject.

Certain standard ventilatory indices were computed for each individual. The one with which this

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paper will deal is the maximal midexpiratory flow (11), which expresses the mean flow rate of the middle half of a forced expiration. It has been found to be a more sensitive index of airflow impairment than the maximal breathing capacity or the one-second or three-second fraction of the vital capacity (11, 12); in general, the maximal midexpiratory flow correlates rather well with the one-second volume of a forced expiration (the $FEV_{1.0}$) (13). Values for the maximal midexpiratory flow were considered abnormal if they were less than half the mean value found for a particular subject's age group. "Normal" values were obtained using data from persons in the survey who had neither respiratory symptoms nor a history of any major chronic pulmonary disease; they are shown in table 1. The use of these values was considered preferable to the use of values based on a smaller sample and reported in Leuallen and Fowler's original article (11). It was realized that the absence of respiratory symptoms was not necessarily an indication of normal ventilatory mechanics and that errors resulting from this technique would, of course, tend to give falsely low "normal" values.

Physical examination was omitted because of time and space limitations and because it was thought that it would add little to this type of study. It is well known that demonstration of physical signs of chronic bronchitis or emphysema depends to a large extent on the individual observer. Chest roentgenograms were not made because most of the subjects had been examined during the previous year and because it was impractical at the time of the study to obtain new films. The official interpretations of recent roentgenograms were not available.

TABLE 1

MAXIMAL MIDEXPIRATORY FLOW IN "NORMAL" SUBJECTS

Age group	Number		MMF(L./sec.)	
	Men	Women	Men	Women
40–49	19	27	4.35 ± 1.53	3.54 ± 0.93
50–59	29	26	3.89 ± 1.37	2.83 ± 0.76
60–69	13	6	3.10 ± 1.30	2.73 ± 0.70
More than 70	9	—	2.98 ± 1.07	

TABLE 2

RESPIRATORY SYMPTOMS IN 172 WORKING ADULTS

	95 Men	77 Women
Cough more or less every day	17 (18%)	13 (17%)
Cough attributed in part to smoking	10	9
Bring up phlegm some mornings	31 (33%)	21 (27%)
Wheeze at times	30 (32%)	20 (26%)
Family history of asthma or bronchitis	19 (20%)	33 (43%)
Chest cold each of past two winters	33 (35%)	29 (38%)
Saw physician past year for chest trouble	8 (8%)	14 (18%)

TABLE 3

MAXIMAL MIDEXPIRATORY FLOW (MMF) IN LUNG DISEASE SUSPECTS

Category	Sex	Number	Mean MMF (L./sec.)
Symptoms only	M	13 (14%)	3.00
	F	14 (18%)	3.30
Impaired MMF only	M	7 (7%)	1.55
	F	2 (3%)	1.50
Symptoms and imparied MMF	M	11 (12%)	1.08
	F	4 (5%)	1.01

RESULTS

The prevalence of certain respiratory symptoms is shown in table 2.

The presence of chronic cough (defined as cough more or less daily for several months) was considered an important symptom, especially if expectoration occurred and if the sputum was sometimes discolored.

Subjects were placed in the following categories: (*1*) no significant symptoms; (*2*) respiratory symptoms without impairment of the maximal midexpiratory flow; (*3*) impaired maximal midexpiratory flow in the absence of symptoms; and (*4*) respiratory symptoms with impaired maximal midexpiratory flow. Sixty-four men (67 per cent) and 57 women (74 per cent) had neither symptoms nor ventilatory performance to suggest the possibility of chronic nonspecific lung disease. Thirty-one men (33 per cent) and 20 women (26 per cent) were suspected of having bronchopulmonary disorders. The mean values of the maximal midexpiratory flow in subjects in categories (*2*), (*3*), and (*4*) above are shown in table 3.

Twenty-seven persons (13 men and 14 women) had various respiratory symptoms but no obvious ventilatory impairment. Their mean ages were fifty-six and fifty years, respectively. Almost all had chronic cough with expectora-

TABLE 4
SMOKING HABITS IN 172 ADULTS

Category	95 Men	77 Women
Lifelong nonsmokers	13 (14%)	36 (47%)
Present or past cigarette smokers	69 (73%)	41 (53%)
Present cigarette smokers	42 (44%)	35 (45%)
20-plus pack-year smokers	35/42	17/35
Ex-cigarette smokers	27 (28%)	6 (8%)
Pipe or cigar smokers	13 (14%)	—

TABLE 5
PRESENT SMOKING HABITS AND RESPIRATORY SYMPTOMS

	Lifelong Nonsmokers		Regular Cigarette Smokers		Pipe or Cigar Smokers
	Men	Women	Men	Women	Men
Number	13	36	42	34	13
Number with					
daily cough	0	2	12	9	0
sputum	1	9	14	12	2
wheeze	1	0	14	8	4
chest colds	2	15	17	12	7

tion. Eleven of the 13 men were moderately heavy or heavy cigarette smokers who had smoked 20 or more pack-years,[4] as had 6 of the 14 women. Only 3 subjects, all women, stated that their exercise tolerance was less than normal for their age and sex.

Nine persons had what was judged to be impaired ventilatory function in the absence of respiratory symptoms. All were included in the so-called "normal" groups for purposes of determining the mean maximal midexpiratory flow for the various age groups. Of the 7 men (mean age sixty-four) in this category, 5 had maximal midexpiratory flow of less than 1.5 L. per sec. Five of the seven were cigarette smokers.

Fifteen subjects had respiratory symptoms and impaired ventilatory function. The mean age of the 11 men was sixty-one and of the women fifty-one years. Thirteen of the 15 had chronic cough, and 10 produced sputum more or less consistently. Thirteen had smoked in excess of 20-pack-years; only one was a nonsmoker. Nine said they had had chest colds during each of the past two winters; 7 of these had seen physicians during the past year because of respiratory symptoms. Five considered their exercise tolerance less than normal. The maximal midexpiratory flow exceeded 1.15 L. per sec. in only one of the 15 subjects.

[4] A pack-year is defined as the smoking of one pack of cigarettes per day for one year or its equivalent, e.g., one-half pack per day for two years.

Persons with Chronic Cough

Seventeen men (18 per cent) and 13 women (17 per cent) complained of more or less daily cough for a period of at least several months. About two-thirds of these 30 persons attributed their cough in part to smoking. More than three-fourths had smoked 20 or more pack-years. Most of the subjects said their cough was productive of phlegm or mucus even in the absence of colds. The incidence of pneumonia or chest colds was no greater in persons with chronic cough than in those not subject to chronic cough. Six of the 30 admitted to less than normal exercise tolerance; eight of 142 without cough made the same admission. A family history of bronchitis or asthma was quite common in the women coughers (8 of 13) but rare in the men (only one of 17); women perhaps are more aware of the family medical history than are men. Men with chronic cough and expectoration had impaired ventilatory function compared with men with no cough or sputum (mean maximal midexpiratory flow 1.90 L. per sec. and 3.73 L. per sec., respectively). In women the difference was not significant (2.77 L. per sec. and 3.18 L. per sec., respectively).

Smoking Habits

The smoking habits of the 172 subjects are summarized in table 4. The figures indicate that the group contained a much higher percentage of smokers than is found in the general population, especially among the women (14). A significant number of men were classified as ex-cigarette smokers, suggesting the possibility that, among the men studied, a considerable number may have stopped smoking because of ill health. Unfortunately, there was no systematic inquiry as to why these persons ceased smoking.

The incidence of respiratory symptoms in lifelong nonsmokers, persons regularly smoking cigarettes at present, and pipe or cigar smokers is shown in table 5. Among men who smoked

cigarettes regularly, chronic cough with expectoration was significantly more common than in nonsmokers or pipe or cigar smokers (p value less than 0.01); women who had smoked twenty or more pack-years had significantly higher incidence of cough with expectoration than nonsmokers (p value less than 0.05). In both men and women the incidence of chest colds was not significantly different in nonsmokers and regular cigarette smokers. Only 2 of 49 lifelong nonsmokers complained of chronic cough. Ventilatory function data in nonsmokers and smokers are contrasted in table 6. Male nonsmokers had significantly higher values than the smokers (p value less than 0.001 for maximal midexpiratory flow). On the other hand, no significant differences in ventilatory function data were found in the women nonsmokers and smokers.

Persons Who Sometimes Wheeze

Thirty men and 20 women said they sometimes wheezed; this was usually described as occurring during the course of a cold. Six of the 50 who wheezed had been told at some time that they had asthma. A family history of bronchitis or asthma was significantly more common in those who wheezed than in those who gave no such history (p value less than 0.01). Ventilatory function was somewhat diminished in those with histories of wheezing, but not significantly so.

Persons Subject to Chest Colds

Thirty-three men and 29 women said they had had chest colds each of the past two winters; only 15 of the 62 had actually consulted physicians in the past year because of chest trouble. The impression was given that many were apt to designate as a chest cold something less than actual bronchitis.

Results of Follow-up Questionnaire

Twenty-seven persons suspected of having chronic pulmonary disorders were re-questioned four months after the initial study, using the questionnaire of Fletcher and associates (8). The extent of agreement between their answers to the two sets of questions is shown in table 7. The changes in the second interview were in the direction of an increased incidence of respiratory symptoms. The 9 disabling (necessitating staying at home from work) chest illnesses during the past three years among the 27 re-interviewed accounted for one hundred days of disability.

TABLE 6

VENTILATORY FUNCTION IN SMOKERS AND NONSMOKERS

Number	Sex	Category	Mean Age	Mean MMF (L./sec.)
13	Men	Nonsmokers	56	4.98 ± 1.48
54	Men	20-plus pack-year smokers	56	2.86 ± 1.37
36	Women	Nonsmokers	51	3.16 ± 0.94
22	Women	20-plus pack-year smokers	49	2.98 ± 1.38

TABLE 7

AGREEMENT BETWEEN FIRST AND SECOND INTERVIEWS

	First	Second
Daily cough	16 of 27	20 of 27
Cigarette cough	13	13
Produce phlegm in absence of colds	13	18
Usually bring up phlegm from chest	—	15
Chest cold each of past two winters	13	11
Disabling chest illness past three years	—	9
Family history of asthma or bronchitis	10	11
Decreased exercise tolerance	5	6
Wheeze at times	11	13

DISCUSSION

There is no particular reason to believe that this group of 172 adults is wholly unrepresentative of persons more than forty in the field of banking and finance, allowing for the fact that about one-fifth of potential subjects did not participate in the study. No information is available regarding those who refused or were unable to take part.

Thirty men and women (17 per cent of the total) complained of rather chronic cough, usually with expectoration. A large percentage of these persons probably have chronic bronchitis. The results of this study would seem to indicate an incidence of bronchitis of about the same magnitude as that found by Pemberton (9) in 369 men aged forty-five to sixty-four employed in engineering and factory work in the eastern United States; nor are the figures in the present study too dissimilar to those of Higgins (15),

who found a 15 per cent incidence of cough and expectoration in Welch agricultural workers older than forty-five.

The association of smoking, respiratory symptoms, and ventilatory impairment has been recorded frequently (6, 7, 12, 15–19). The present study re-confirms this in men and points out the low incidence of symptoms and of ventilatory impairment in lifelong nonsmokers in this particular group of subjects. On the other hand, the data in the present study indicated no significant loss of ventilatory function as measured by the maximal midexpiratory flow in women who had smoked for many years, despite the fact that respiratory symptoms were more common in them than in nonsmokers.

Two interesting groups of persons with respiratory symptoms were noted in this survey: those with symptoms only and those with symptoms and obvious ventilatory impairment. Whether the factors accounting for ventilatory impairment in the latter group are simply quantitative, i.e., more prolonged cough, bronchial inflammation, et cetera, is not apparent from the present study.

Summary

A survey to detect chronic bronchitis and asthma-like conditions is reported upon.

One-hundred and seventy-two adult men and women more than 40 years old, employed in a bank, were questioned regarding respiratory symptoms. Spirograms were obtained, and the maximal midexpiratory flow was computed for each subject.

Forty-two persons (roughly 24 per cent of the total) had respiratory symptoms suggesting the likelihood of chronic pulmonary disease. Ventilatory function in 27 of these persons was not impaired. In 15 persons, there was evidence of impaired ventilatory function.

Men with chronic cough and expectoration had a significantly lower mean maximal midexpiratory flow than men without cough and expectoration. Prolonged cigarette smoking was closely associated with chronic cough and expectoration. Ventilatory function in male nonsmokers was significantly better than in smokers; no significant difference in ventilatory function was found between women nonsmokers and smokers.

Sumario

Síntomas Respiratorios y Función Ventiladora en 172 Adultos Empleados en un Banco

El estudio descrito se proponía descubrir bronquitis y estados parecidos al asma de naturaleza crónica.

Se interrogó con respecto a síntomas respiratorios a 172 sujetos adultos (de más de 40 años de edad) de ambos sexos, empleados en un banco. Se obtuvieron espirogramas y se computó para cada sujeto la corriente mesoespiratoria máxima.

Cuarenta y dos personas (más o menos 24 por ciento del total) tenían síntomas respiratorios que indicaban la probable existencia de neumopatía crónica. En 27 de estas personas no estaba afectada la función ventiladora. En 15, había signos de menoscabo de dicha función.

Los varones que tenían tos y expectoración crónicas mostraron una corriente mesoespiratoria máxima significativamente menor que los que no tenían tos y expectoración. El uso prolongado de cigarrillos se vinculaba íntimamente con la tos y expectoración crónicas. La función ventiladora era decididamente mejor en los varones que no fumaban que en los fumadores; no pudo descubrirse mayor diferencia en la función ventiladora entre las mujeres que fumaban y las que no fumaban.

Resume

Symptômes respiratoires et fonction de ventilation chez 172 adultes, employés dans une banque

L'auteur rapporte un examen pour dépister les conditions de bronchite chronique et de simili-asthme.

Cent - soixante - douze adultes, hommes et femmes, au-dessus de 40 ans, employés dans une banque, furent interrogés à la recherche de symptômes respiratoires. On obtint des spirogrammes, et le débit maximum à la moitié de l'expiration fut calculé pour chaque sujet.

Quarante-deux personnes (à peu prés 24% du total) avaient des symptômes respiratoires faisant penser à la possibilité de maladie chronique pulmonaire. La fonction respiratoire de 27 de ces personnes n'était pas altérée. Chez 15 personnes, il y avait évidence d'une altération de la fonction respiratoire.

Des hommes avec une toux chronique et une expectoration avaient une moyenne de maxima, à mi-expiration, nettement inférieure aux hommes sans toux et sans expectoration. L'état de longue durée, de fumeurs de cigarettes fut en rapport étroit avec la toux chronique et l'expectoration. La fonction respiratoire chez les hommes non-

fumeurs fut nettement meilleure que chez les fumeurs; on ne trouva pas de différence appréciable de la fonction pulmonaire entre les femmes qui fument et celles qui ne fument pas.

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A TECHNIQUE FOR THE STUDY OF VENTILATORY CAPACITY, COMPLIANCE, AND RESIDUAL VOLUME OF EXCISED LUNGS AND FOR FIXATION, DRYING, AND SERIAL SECTIONING IN THE INFLATED STATE[1, 2]

PHILIP C. PRATT AND GEORGE A. KLUGH

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Introduction

During the past three years the writers have been conducting a study of excised normal and emphysematous lungs for the purpose of identifying the morphogenesis of diffuse obstructive emphysema. The procedures for fume fixation, drying, sectioning, and examining of lungs were presented at the American Trudeau (now Thoracic) Society meeting in 1959, simultaneously with those of Blumenthal and Boren (1). It became clear early in the project that distinct changes in structure could be identified in lungs obtained from subjects with no previous significant pulmonary complaints. Since it would be only by the rarest chance that such persons would have had even the simplest pulmonary function studies done before death, it seemed important to develop methods for determining the functional capacity of lungs obtained after death. Indeed, because of the prolonged course of obstructive emphysema, it is quite rare for persons with this disease to die in the hospital and have autopsies performed after having had pulmonary function studies carried out during life. Furthermore, even when such studies are available, there is usually a considerable time lapse between the physiologic studies and the date of death.

It was shown by McIlroy and Christie (2–4) that consistent, and therefore useful, observations concerning pulmonary mechanics can be made with excised lungs. Since many aspects of the methods developed for the present studies are different from theirs, all of the steps involved will be described here. The methods for fixation of lung tissue by formalin fumes, followed by drying in the inflated state for structural studies, are also different from those published previously (1, 5) and will be presented. The various techniques will be presented in sections as follows: construction of respirator box; preparation of excised lungs for study; techniques for functional investigation; fixation and drying of lungs; serial sectioning of dried inflated lungs; examination and dissection of bronchioles and alveolar ducts.

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[2] This study was supported by grants from the Ohio Thoracic Society.

Materials and Methods

1. *Construction of Respirator Box*

The respirator box, a very simple piece of apparatus, can be made from essentially any material as long as it has at least one transparent side for observation of the lung. The box used for the present study is essentially similar to the one used by McIlroy and Christie (2–4). It is made entirely of half-inch thick Plexiglas and measures 16 by 16 by 12 inches (figure 1). This size has been sufficient to permit inflation of even the largest emphysematous lung observed to date without any contact of tissue with the surfaces of the box. The door should be large and quickly removable because repeated handling of a lung may be necessary; window locks have functioned well. The box does not need to be absolutely airtight because it is not necessary for it to maintain a vacuum in the absence of suction. The application of stopcock grease to the facing surfaces of the door has made the box sufficiently leak proof. There should be a number of openings into the box for attachment of apparatus. In the box used in this laboratory there are four openings one inch in diameter, and five, three-eighths of an inch. The larger ones are used for attachment of the bronchus and for apparatus for injection of Latex or other materials into bronchi or vessels. Smaller ones are used for the attachment of the vacuum source, manometer, and electronic apparatus. Unused openings are closed with rubber stoppers.

2. *Preparation of Lung*

All lungs have been obtained from the regular autopsy service of the general hospital of the Ohio State University College of Medicine. In general, one lung from any given case has been used for this investigation while the other lung has been studied by routine methods. For practical reasons, it has not always been possible to conduct the

studies on the day lungs were obtained. Accordingly, all lungs have been quick-frozen and thawed before study and some have been kept frozen for a week or so prior to study. A few tests of some aspects of function, namely expiratory flow and residual volume, have been carried out before and after freezing and have shown no change.

The first step in preparation is the insertion into the hilar bronchus of a connector which should be as large as possible in order not to introduce unnecessary resistance to air flow. Rigid plastic test tubes of various diameters, with the end cut off, are ideal for this purpose. For the ventilatory studies the lung must be entirely airtight. It is rarely possible to remove a lung, particularly an emphysematous one, from the chest without perforating the pleura. However, if care is exercised, the perforations can be kept small enough to be closed without excessive distortion of the tissue. For closure of a perforation, the site is grasped with a forceps and a loop of size 0 black braided silk suture is tied around the opening. Needles are not used because the needle holes establish new sites of leakage. Leakage can be detected by means of a spirometer, and obscure leaks can be identified by immersing the lung in water with air pressure applied to the bronchus. The production of air bubbles will lead to the identification of even the smallest leak. In applying air pressure to the lung for this purpose, care should be taken that it does not exceed 15 to 20 cm. of water, because of the risk of producing artefacts.

3. *Techniques for Functional Investigation*

A. Preliminary inflation of lungs: During removal of lungs from the chest, they are frequently quite forcibly collapsed. In addition, several hours may pass between the time of removal and the time of study. For these reasons, many surfaces within the lung will be apposed, and the responses to inflation pressures under these circumstances are far different from those obtained with a lung which has been recently inflated and has collapsed spontaneously. This phenomenon has been studied in detail by McIlroy (2). Therefore, before functional studies are conducted, the lung is first fully inflated. During this inflation the amount of pressure differential applied is limited to a maximum of 15 to 20 cm. of water. If the lung will not fully expand with this amount of force, it is sometimes necessary to manipulate the few secondary lobules which have resisted inflation.

B. Static inflation and deflation: The static inflation and deflation test is the first test performed on all lungs. In this procedure the lung is suspended in the respirator box with the bronchus connected to a spirometer through large tubing, such as is used in any pulmonary function laboratory. A water manometer is connected to the respirator chamber. Removal of air from the box by connecting it to a source of vacuum will cause the lung to expand. A deep water container with a segment of glass tubing which can be inserted into the water to various depths is placed in the suction line tubing (figure 1). This provides accurate regulation of the amount of suction applied to the box.

The static inflation is carried out by first allowing the lung to collapse freely from its inflated state by removing the pressure regulating tube completely from the water. Then the tube is inserted to a depth of 2 cm. and the lung is allowed ample time to respond to this pressure change; any volume change is recorded by the spirometer. After the volume stops changing, the tube is inserted to 4 cm. and again the volume change is recorded. Continued increments of 2 cm. of pressure are applied until it is noted that the lung no longer increases appreciably in volume (figure 2). *This represents the normal maximal size for a given lung, and the pressure at which the lung reaches this volume should not be exceeded in subsequent tests.* The "static deflation" is done similarly by decreasing the pressure in 2-cm. steps and recording volume changes with the spirometer. During these tests the strain gauge[3] and electronic recording equipment[4] to be used for recording dynamic pressure changes are calibrated with the manometer.

C. Dynamic inspiratory compliance: To perform the dynamic inspiratory compliance test, the lung is first allowed to collapse freely by opening one of the outlets of the respirator box. The pressure-regulating tube is inserted to the depth previously determined by the static inflation test to represent the maximal pressure which should be applied to this lung. The respirator outlet is then closed, and the pressure changes in the box are recorded electronically while the inflation curve is recorded by the spirometer. The rate of inflation is determined by the rate of flow of the suction applied. The rate usually used here has been 250 cc. per second; both higher and lower rates could also be applied. The apparatus records both the pressure and volume changes against time. The pressure then can be plotted against the volume to produce a curve reflecting the changing compliance of the lung during inflation.

D. Expiratory flow rate: The expiratory flow rate is determined by releasing the "negative pressure" within the box suddenly and recording the volume change in the lung against time by means of a spirometer. Various rates of collapse can be produced by varying the size of the opening through which air is allowed to enter the box. Compliance curves can also be made, but the spirometer cannot be used for this purpose because it does not respond sufficiently rapidly. Electronic apparatus with flowmeter[5] and strain gauge are required for expiratory compliance curves.

E. Residual volume: For determination of residual volume, the lung is permitted to collapse

[3] Statham Pressure Transducer, Statham Laboratories, Los Angeles, California.

[4] Sanborn Polyviso with Strain Gauge Amplifier, Sanborn Company, Cambridge, Massachusetts.

[5] Vol-O-Flo Respiratory Flowmeter, National Instrument Laboratories, Washington, D. C.

freely from the fully inflated state for ten minutes. The box is then opened and a stopper is placed in the connector in the bronchus so that handling will not cause further escape of air. The lung is then removed from the box and immersed in water and the displaced water is measured. The weight of the lung in grams is subtracted from this volume, and the difference is considered to represent the amount of air remaining in the lung. This is defined as the residual volume for purposes of this study. It obviously has no specific relationship to the residual volume obtained during life, but still this volume has consistently been higher for emphysematous lungs than for normal ones. Residual volume can also be measured by standard methods such as helium dilution (2, 6). In a number of such tests there has been fairly good correlation between the volume obtained by water displacement and that obtained by helium dilution.

4. *Fixation and Drying*

An airtight lung will not fix if a steady pressure of formaldehyde fumes is simply exerted upon it. Accordingly, a method is used to cause the lung to aspirate or "breathe" the formaldehyde fumes. For this purpose, the tube from the bronchus is connected to a 500-ml. Erlenmeyer flask containing 150 ml. of formaldehyde which is warmed on a hot plate to about 60° C. The lung is made to "breathe" by means of a ported cylinder and piston illustrated in figure 3. During fixation the maximal pressure applied is still limited to that found to be correct for the lung. A one-way valve is placed in the tubing from the formaldehyde bottle to the lung so that with each inflation new formaldehyde fumes are aspirated into it. The lung tissue will fix in twelve to eighteen hours. Although fixed, the lung tissue is still quite elastic and will collapse and become distorted if sectioned. In order to prevent this, the lung must be dried in the inflated state.

The lung is dried by continuous application of pressure at the previously determined maximum for some five to eight days. During this period air diffuses through the pleura, and moisture from the tissue evaporates into this air. Beginning at five days, the lung is weighed daily and, when the weight ceases to decrease, the lung is considered to be completely dry and can be permanently removed from the box.

5. *Serial Sectioning of Dried, Inflated Lungs*

The lung is most satisfactorily examined by the preparation of numerous thin slices. This can best be accomplished by means of a band saw equipped with a double-bevel, straight-edged knife blade. The guide mechanism used here is shown in figure 4. For normal lungs, sections can be prepared in the range of 1 mm. in thickness, but in the presence of emphysema thicker sections must be cut because thin ones will not hold together sufficiently for study.

6. *Examination and Dissection of Bronchioles and Alveolar Ducts*

The most informative areas for evaluation of pulmonary structure are those in which bronchioles and related alveolar ducts have been cut in longitudinal section. By cutting a number of serial sections one can always be certain that there will be several such areas available for study in each lung prepared. Frequently it will be found that a bronchiole is parallel to the cut surface but just below it or a bronchiole may dip in and out of the surface. In such instances it is possible to dissect off the overlying tissue and reveal the structures. Highly satisfactory dissecting scissors can be made from rubber tubing and two straight-edged disposable surgical scalpel blades (figure 5).

Results

Detailed results of the studies carried out by the above methods will be presented in subsequent publications. A typical static inflation-deflation curve for a normal lung is shown in figure 2. Note that there is little change in volume as the pressures change from 0 to 2 and 4 cm. of water. At 6, 8, and 10 cm. there are substantial volume changes, but at 12 and 14 cm. the changes become insignificant. The lung has obviously reached its maximal normal size. Emphysematous lungs characteristically reach their maximal size at pressures of 8 to 12 cm.

The arrangement of a bronchiole with related alveolar ducts and alveoli in a "normal lung" is illustrated in figure 7. This lung is considered normal because it was obtained from a young person (without pulmonary complaints) killed in an auto accident and because its response to static inflation and deflation, its compliance, expiratory rate, and residual volume fell within the range of those found with other "normal lungs." In addition, the appearance of the pulmonary tissue seen in multiple serial sections was uniform throughout. An area observed in a lung from an older person is shown in figure 8. In this instance the postmortem function studies also fell within the range of normal. Two alveolar ducts are seen branching from a bronchiole; one of these is of normal size and distribution while the other opens directly into a small bulla.

Discussion

It is hoped that the present description of methods for functional studies of excised lungs

will enable others to pursue similar projects. It is already clear that even the most elaborate structural studies may be impossible to interpret if one does not have some objective knowledge of the functional capacity of the lungs examined. While the functional studies described here are quite simple, it will be shown in subsequent publications that excised emphysematous lungs have greater total volume, greater compliance, greater residual volume, and slower expiratory flow than normal lungs.

Previous writers, especially McIlroy (2) and McIlroy and Christie (3, 4) have shown that, in order to obtain consistent and comparable data for the volume or compliance of excised lungs, it is necessary to conduct the measurements after the lung has previously been inflated under standard conditions. This principle has been followed in each of the tests described here. Those writers used an arbitrary maximal pressure reduction of 10 cm. of water for inflation and measured the volume of air remaining in the lung after it was subsequently returned to a more nearly atmospheric pressure. The data obtained cannot be correlated with any volumes measurable during life. In the present study, the proper maximal pressure to be exerted was determined for each lung (Technique 3, *B*) and the amount of air present in the lung at full inflation can be ascertained by measuring the volume of air expelled as the lung collapses and adding to this the residual volume (Technique 3, *E*). The resultant data for total lung volume are more informative than McIlroy's figures because they can be compared with values for total volume determined during life, or predicted according to standard formulas.

The importance of determining the normal maximal size of each lung and the pressure necessary to achieve this volume should again be stressed. These characteristics are revealed by the static inflation curve illustrated in figure 2. The pressure determined by this means should not be exceeded in subsequent tests because of the risk of producing artefacts in the lung sections. Such artefacts usually occur first in the perivascular and peribronchial areas in which loose areolar tissue is stretched and torn. When this has occurred, these structures, as seen in the sections of the dried lung, will appear widely separated from adjacent pulmonary tissue. Other artefacts include the accumulation of air in secondary lobular septa and beneath the pleura. When such changes are present, it becomes impossible to rely upon observations in the structure of alveolar ducts and alveoli. It may be re-emphasized that the pressure required for maximal inflation of an emphysematous lung is usually several centimeters of water less than that required for normal lungs.

A question frequently asked during discussions of this project was whether the lung should be inflated by exerting positive pressure through the bronchus or by being placed in a box and applying "negative pressure." In either case the lung actually inflates only because of a transpleural pressure differential, and the intrapulmonary mechanical situation is identical. The only reason for using a respirator box is that this permits attachment of the bronchus to a spirometer for the purpose of volume measurements. Such measurements would be difficult to achieve if the lung were being inflated by intrabronchial pressure.

Summary

Methods have been described for the determination in excised lungs of the total volume, compliance, residual volume, and expiratory flow rate, as well as for the fixation and drying of lung tissue in the inflated state and for serial sectioning of the dried lung. It is emphasized that functional studies should be conducted on lungs which are to be the subject of elaborate structural investigations.

Sumario

Técnica para el Estudio de la Capacidad Ventiladora, del Acomodamiento y del Volumen de los Pulmones Excindidos, y para la Fijación, la Desecación y la Obtención de Cortes Seriados en Estado de Inflación

Los métodos descritos sirven para la determinación en los pulmones excindidos del volumen total, del acomodamiento, volumen residual y velocidad de la corriente espiratoria, así como para la fijación y desecación de los tejidos pulmonares en estado de inflación y para la obtención de cortes seriados del pulmón desecado. Recálcase que deben llevarse a cabo estudios funcionales en pulmones que van a ser objeto de minuciosas investigaciones histológicas.

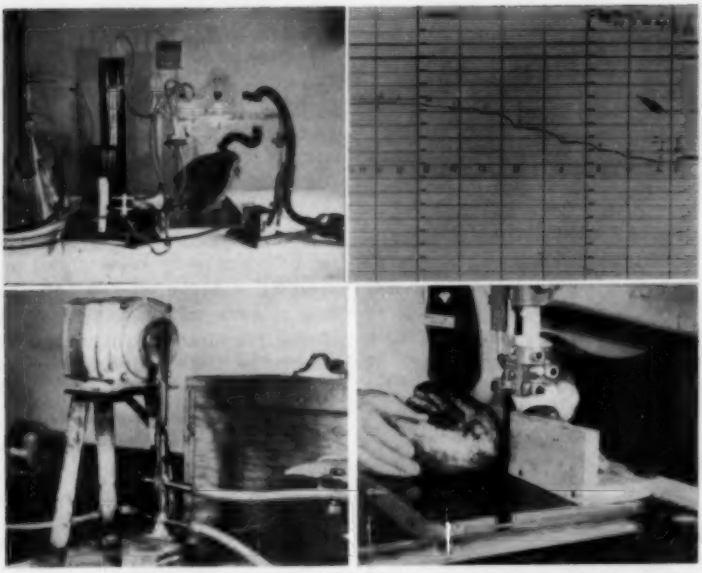

FIG. 1 (Upper left). Respirator box and associated apparatus used for functional studies. At the far right is tubing extending from the large opening in the box to a spirometer. Inserted in this tubing are a flow meter and strain gauge used for recording flow rate. Similar large tubing extends from the box opening to the connector in the bronchus. Note that the connector is of the largest size which can be inserted into the lumen of the hilar bronchus. The remaining large openings into the box are closed by stoppers. Tubing extends from the smaller openings to various pieces of apparatus, including a manometer at the top and a strain gauge for recording pressures in the box at the bottom of the picture. The two remaining tubes extend to the apparatus used for causing the lung to "breathe" (*see* figure 3). Rubber tubing extends from this apparatus to the pressure-regulating bottle and from there to a suction trap which is the smaller flask at the right of the large one and finally from the trap to the primary source of suction. The pressure-regulating tube is open to the air at the top of the regulating bottle. This tube is adjustable but in the illustration extends 10 cm. below the surface of the water in the bottle, and the manometer indicates the existence of 10 cm. of "negative pressure" within the box. The lung is inflated.

FIG. 2 (Upper right). Static Inflation curve. This photograph shows the spirometer tracing obtained during a static inflation of a normal lung. The tracing extends from right to left. The zero at the right (arrow) indicates the presence of atmospheric pressure within and outside the respirator box. At the points indicated by 2, 4, 6, et cetera, the pressure in the box has been diminished by 2, 4, and 6 cm. of water. The response of the lung to the application of these pressures is revealed by the step-wise increase in volume. Note that the major increases in volume occurred when the pressure was changed from 6 to 8, 8 to 10, and 10 to 12 cm. From 12 to 14, 16, and 18 cm. the volume changed but little. The higher pressures were applied only to clearly demonstrate that the lung had reached its essential maximal volume at 12 cm.

FIG. 3 (Lower left). The ported cylinder and piston apparatus with motor for causing lungs to "breathe" during fixation. The electric motor is equipped with a reducing gear so that the shaft rotation is 6 per minute. Note the opening drilled through the piston. When the piston is down, its end occludes the lower port extending from the cylinder and interrupts the suction source. At the same time the opening in the piston lines up with the upper port and allows air to enter the box. The lung collapses because of its elasticity. As the piston rises, the upper port is closed and the lower one is opened and suction causes re-expansion of the lung.

FIG. 4 (Lower right). Band saw arranged for serial sectioning of dried inflated lung. Note the strip of wood clamped to the saw table at the far right. The guide piece or fence slides against this clamped piece which is positioned so that the space between the fence and knife blade will provide the thickness of section desired. The lung is held against the fence and both are slid past the blade.

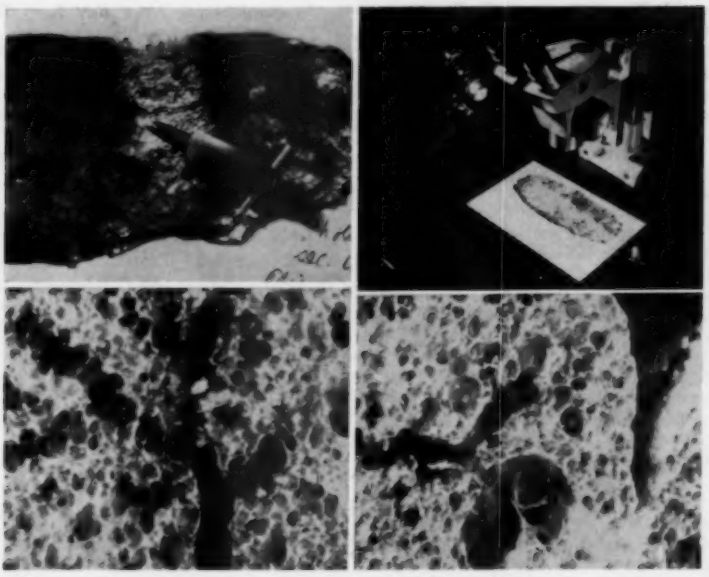

FIG. 5 (Upper left). Dissecting scissors for bronchioles and alveolar ducts. The scissors are made from two straight-edged disposable scalpel blades. The blades are inserted through a short segment of three-eighths inch rubber tubing and another piece of tubing is inserted between the blades at the heel. A fragment of applicator stick is placed through the latter piece of tubing to exert slight tension on the blades. Pinching the blades at the heel results in a scissors action of the points. Under the dissecting microscope at the magnification of 10×, dissections are quite easily accomplished.

FIG. 6 (Upper right). Arrangement for photography with dissecting microscope. Transmitted light and vertical surface lighting are unsatisfactory for photographing the thin, almost transparent membranes of pulmonary tissue. A low tangential light, as shown here, provides the degree of contrast needed for photography. It is usually necessary to provide a lens shade for the objective lens of the microscope because of undesirable lens reflection.

FIG. 7 (Lower left). Normal relationship of bronchioles and alveolar ducts. A bronchiole enters the field from the lower center. An alveolar duct branches from this toward the right, and the bronchiole then becomes a respiratory bronchiole with alveolar spaces opening directly from its lumen. Further branchings are also alveolar ducts. (15×) (reduced ½)

FIG. 8 (Lower right). Bronchiole, alveolar ducts, and emphysematous bleb. The bronchiole in this field has been cut in cross section at left center. It branches into a respiratory bronchiole which promptly branches into two alveolar ducts, the upper one of which is of normal size and distribution while the lower one opens directly into a distinct emphysematous bulla. (15×) (reduced ½)

RESUME

Une technique pour étudier la capacité de ventilation, l'extensibilité, et le volume résiduel de poumons extirpés, et pour fixer, sécher et faire des coupes en séries lorsqu'ils sont collabés

Les auteurs décrivent des méthodes pour déterminer le volume total de poumons extirpés, leur extensibilité, le volume résiduel, et la valeur du débit expiratoire, en même temps que des méthodes de fixation, de séchage du tissu pulmonaire collabé et de coupes en séries du poumon asséché. Ils insistent sur le fait que les études fonctionnelles devraient être menées sur des poumons qui doivent subir des études compliquées de leur structure.

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FAMILIAL EMPHYSEMA[1]

FELIX WIMPFHEIMER AND LOUIS SCHNEIDER

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INTRODUCTION

Well-documented instances of familial emphysema of the lungs have not been recorded in the American medical literature. By contrast, Hurst (1), in a casual survey of the world's literature from the beginning of the nineteenth century to the present, has encountered the recurrent notion that a familial predisposition to this disturbance does exist. He refers to a British text by West (2), written in 1902, in which, according to Lebert (3), thirteen of 108 patients showed a familial pattern. In the same volume, Schnitzler (4) is quoted as having observed three brothers, "who became emphysematous without obvious cause." Roessle (5), in a German treatise on medical genetics, published in 1940, collected 31 examples of familial emphysema proved by autopsy. He writes: "It is astonishing that in texts of human genetics up to now, the question of heredity in pulmonary emphysema has been neglected." The surprisingly large number of cases he was able to collect convinced him that the lung was one of the organs most strongly affected by hereditary factors. Hurst (1), in his article published in 1959, reports briefly on two families in which three and five brothers, respectively, were affected by pulmonary emphysema. In the same year, Despierres and associates (6) reported encountering bullous emphysema in two and perhaps three siblings.

The purpose of this communication is to report in some detail on two more families in which several members were affected by emphysema of the lungs and to draw renewed attention to the possible role played by constitutional factors in the pathogenesis of pulmonary emphysema.

CASE REPORTS

Family A

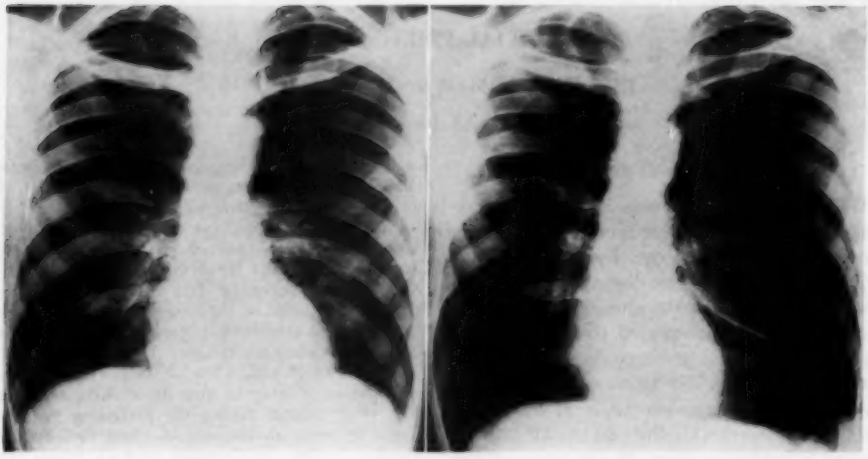
Case 1. L. S., a Negro male, born November 10, 1917, was hospitalized in September, 1950, at the age of thirty-three because of a septic condition characterized by fever, sweating, shaking chills, and weight loss. His sputum was negative for acid-fast bacilli. The blood test for syphilis was positive. A roentgenogram of his chest revealed extensive bullous emphysema involving both upper lobes of his lungs. Pulmonary function studies showed slight impairment of his lung volumes. In September, 1951, he presented himself to the chest clinic of the Washington Heights Health Center, Department of Health, City of New York, with the complaints of fever of nine days' duration, weight loss of 20 pounds during the preceding year, and slight dyspnea on exertion. A chest film disclosed marked bullous emphysema of both upper lobes (figure 1).

The patient's condition deteriorated progressively during the next four years, as indicated by increasing shortness of breath, impairment of his pulmonary functions by objective measurement, and evidence of enlargement of the bullae on the roentgenogram. Roentgenograms of the chest in 1955 showed giant bullae occupying the upper two thirds of the left lung and compressing the remaining lung parenchyma on that side. Less advanced bullous emphysema was seen in the upper third of the right lung (figure 2). In September, 1955, giant bullae were excised from the left lung. The patient has felt markedly improved since the operation and postoperative roentgenograms have confirmed re-expansion of lung tissue previously compressed.

Case 2. L.S., a Negro female, born January 15, 1920, and a sister of the patient of *Case 1*, was seen at the Washington Heights Health Center for the first time on October 24, 1952, because of a slightly productive cough of three weeks' duration. A roentgenogram of her chest revealed the presence of marked bullous emphysema in the upper half of her left lung and the uppermost fifth of her right lung (figure 3). A follow-up examination on November 5, 1959, disclosed considerable weight loss but no respiratory complaints. A roentgenogram of the chest at this time showed marked progression of the emphysematous process which was seen to involve the greater portion of the lung fields on both sides (figure 4).

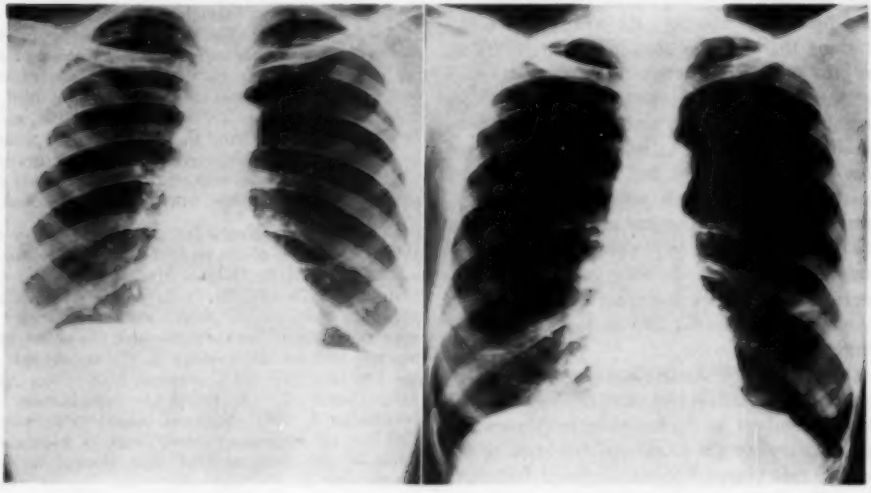
Survey of family A: A chest roentgenogram of the patients' father was reported to be normal. Despite strenuous efforts, it was impossible to convince the patients' mother to submit to a roentgenogram of her chest. The chest films of the remaining siblings, a brother *C.S.* and a sister *F.L.*, were found to be normal.

[1] From the Medical Division, Montefiore Hospital, New York, New York.



Case 1

FIG. 1 (Left). Chest film obtained in 1951 showing severe bilateral bullous emphysema.
FIG. 2 (Right). Chest film obtained four years later (1955), demonstrating marked progression of the bullous process, especially on the left.



Case 2

FIG. 3 (Left). Chest film of sister of patient of *Case 1*, obtained in 1952, showing bilateral bullous emphysema.
FIG. 4 (Right). Chest film obtained in 1959, seven years later, showing marked progression on both sides.

Family B

Case 3. P.C., a white bank clerk, noticed the onset of shortness of breath at the age of thirty-five. The dyspnea was progressive and was eventually complicated by cough, the production of purulent sputum, and right heart failure. At the age of forty-one pulmonary emphysema was diagnosed. Two years thereafter, in 1948, the patient was admitted to the University of Chicago Clinics.

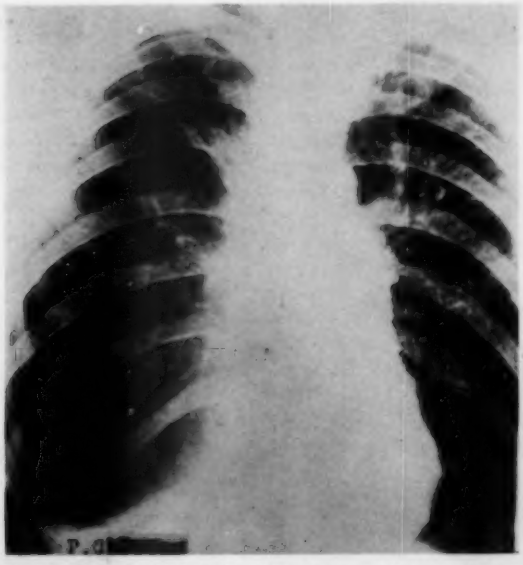


FIG. 5. Chest film of *Case 3*, obtained in 1948, demonstrating marked emphysema, more advanced on the right, and prominence of the pulmonary arterial segment.

Physical examination revealed the signs of pulmonary emphysema and excessive activity of the accessory muscles of respiration. There was no evidence of bronchial obstruction. Moist rales were heard at both lung bases, and the liver was enlarged. Roentgenograms of the chest (figure 5) showed severe pulmonary emphysema bilaterally, more on the right. The vital capacity measurements were 1.1, 1.3, and 1.4 liters, respectively, representing 27 to 32 per cent of normal. After discharge from the hospital the illness continued its relentless course of progressive pulmonary failure. Death occurred on January 26, 1949. The autopsy showed extensive emphysema in both lungs, more pronounced on the right.

Case 4. R.C., a younger brother of the patient of *Case 3*, developed a chronic cough in 1940. In 1947 and in 1948 he experienced attacks of dyspnea, the first one while painting his home with a sprayer and the second one during the use of a soldering iron. From the latter episode onward, he has experienced dyspnea on exertion. In 1949 he complained, in addition, of the daily expectoration of one-half cup of white to yellowish sputum. His vital capacity at that time was 4.1 liters and the roentgenogram of his chest (figure 6) showed increased translucency of both lungs and flattening of the diaphragmatic leaves. On October 7, 1950, the vital capacity was 3.05 liters. At no time was there clinical evidence of bronchial obstruction.

Case 5. G.D., a sister of the patients of *Cases 3* and *4*, was first observed to have pulmonary emphysema about ten years ago at the age of forty-five. At present her condition is in a far advanced state. Pulmonary functions tests performed in April, 1960, were as follows: Vital capacity, 2.1 liters; first-second vital capacity, 30 per cent of the total vital capacity; midexpiratory flow rate, 0.22 seconds; alveolar nitrogen after seven minutes of oxygen breathing, 9.5 per cent; resting arterial oxygen saturation, 96 per cent; arterial oxygen saturation after exercise of 25 steps, breathing room air, 92 per cent. These data are in keeping with severe air trapping in the lungs.

Survey of family B: Dr. Benjamin Burrows discovered other members of this family with bronchopulmonary disturbances. One other brother was diagnosed as having pulmonary emphysema. The maternal grandmother and her sister had chronic bronchitis. There are two female cousins and one male cousin on the maternal side with pulmonary emphysema. Of nine children, born to the three brothers and one sister, one boy has recurrent bronchitis.

COMMENT

Emphysema and bullous emphysema refer to distention of the distal air spaces of the lung, varying in extent and accompanied by differing degrees of tissue destruction, especially loss of

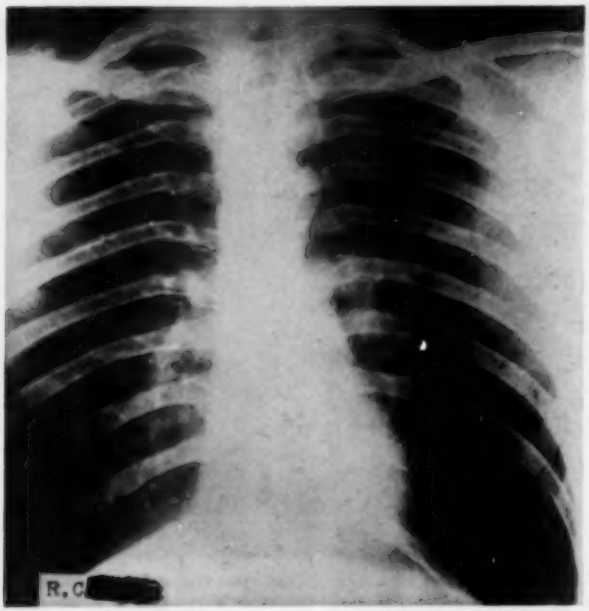


FIG. 6. Chest film of *Case 4*, obtained in 1949, showing diffuse emphysema especially marked in the lower portions of both lungs. The patient of *Case 4* is the brother of the patient of *Case 3*.

the elastic tissue component. This lesion may be localized, multiple, and diffuse. In accordance with recently expressed opinion, it is generally considered as the pathologic end result of many different causative factors and multiple pathogenetic pathways. Mitchell (7) mentions the following etiologic categories: chronic recurrent bronchiolar infection, severe coughing, inflammatory swelling of the bronchiolar mucosa with impairment of ciliary action, premature aging or some other derangement of pulmonary or bronchial elastic tissue or the tracheobronchial cartilage, primary disorders of the pulmonary or bronchial circulation, structural defects of the thorax, and the possibility of an hereditary tendency. In the same symposium, Liebow (8) enumerates traction, diffuse or focal obstruction of air passages, and destruction and loss of elasticity as pathogenetic mechanisms. It bears emphasis that the generalization expressed especially by Amberson, Spain, Kaufman and McLean (9–11) that focal bronchiolitic obstructive changes are the cause of diffuse obstructive or multicentric bullous emphysema is not accepted by all students of this group of affections.

There are several lines of evidence pointing to the importance of weakness of the tissues distal to the terminal bronchiole, possibly on a constitutional basis, as contributing to the pathogenesis of emphysema. As mentioned in the introduction, a familial incidence of pulmonary emphysema has been observed on several occasions. According to Liebow (8) and Fishman (12), Marfan's syndrome, a condition characterized by a constitutional defect in connective tissue, is at times featured by nonobstructive pulmonary emphysema. In addition, the Ehlers-Danlos syndrome and cutis laxa, other heritable disorders of the connective tissue, have been found to be associated with pulmonary emphysema, sometimes of severe degree (13). Bullous emphysema is reported to be prevalent among North African Negroes (14). Finally, autopsied cases of emphysema of the lungs failed to reveal abnormalities of the bronchiolar mucosa (15).

It is also conceivable that constitutional factors act via obstructive mechanisms in the terminal bronchioles. Thus, mucous plugging in the bronchioles is thought to be at work in the pro-

duction of the emphysematous lung found in mucoviscidosis. Moreover, functional obstruction has been postulated to occur due to an increased tendency for collapse of weakened bronchiolar walls during expiration (16). In addition, the possibility exists that functional obstruction is produced by pressure of tense bullae on neighboring bronchiolar walls, especially if these walls are frail (9).

In the recent German literature pulmonary and bronchial arterial insufficiency of varied origin has been implicated as a cause for or accompaniment of the phenomena of atrophy of the lung characterized by emphysema (15, 17, 18). It is of interest that, in unilateral emphysema, pulmonary arterial atrophy is frequently observed on the involved side. The possibility exists that genetic control is exerted through this modality.

Several observers have stated their impressions that an inherent weakness of the lungs in certain persons makes them especially prone to the development of emphysema in response to noxious stimuli (16, 19). Thus, a given degree of bronchiolar obstruction or increase in intra-alveolar pressure of other origins may lead to emphysema in some persons and not in others. The fact that in 4 of the patients described in this communication pulmonary emphysema was discovered in the fourth decade is compatible with the assumption that their lung tissues were especially susceptible. Diffuse obstructive emphysema is usually discovered at a later age.

Both diffuse obstructive and bullous emphysema are more frequently found in the male. A report by Price and Teplick (20) on eight cases of vanishing lung includes no females. Fifty-six cases of bullous emphysema have been observed in the chest clinic of the Washington Heights Health Center, with a ratio of male to female of 50 to 6. That one of two siblings in family *A* and three of seven members with known emphysema in family *B* were female permits the inference that the genetic factor at work has outweighed the factor of sex. The potential presence of a common environmental noxious stimulus must be considered. Such a factor, however, was not uncovered.

The type of progressive bullous emphysema seen in *Cases 1* and *2* has been called "vanishing lung" (21). Price and Teplick (20) expressed the opinion that this is a disease *sui generis* and that it should be differentiated from other varieties of bullous emphysema. It is noteworthy that the course of multicentric bullous emphysema may be quite variable. A minority of cases go on to disintegration of the lungs with progressive respiratory failure. Others remain stationary and asymptomatic for prolonged periods of time. In contrast to patients with diffuse involvement with so-called obstructive pulmonary emphysema, who seek aid because of a variety of respiratory symptoms, the patient with fairly widespread bullous disease in his lungs is often discovered on routine roentgenographic surveys. This is probably due to the fact that the bullae are superimposed on sufficiently healthy lung tissue to support normal respiratory function.

The following classification is presented to place the various forms of pulmonary emphysema into clinical perspective:

1. Obstructive (diffuse):
   - *A) Lobar:* Usually found in infancy and childhood, often traceable to anomalous bronchial or vascular supply of the affected lobe. Treatment: lobectomy. No recurrence.
   - *B) Unilateral:* One whole lung is involved; the other lung is normal in structure and function. This condition is often discovered on a routine chest film in a young adult. Anomalous pulmonary circulation is present and is well demonstrated by pulmonary angiography. No treatment.
   - *C) Bilateral:* The most commonly encountered form of pulmonary emphysema. Etiology is variable and sometimes undetermined. It is usually progressive and in later stages may be accompanied by the appearance of bullae. Pulmonary insufficiency, pulmonary failure, and cor pulmonale are common sequelae. Treatment is symptomatic.
2. Bullous
   - *A) Isolated or unilocular:* This is frequently the end result of pulmonary infection. In infancy and young childhood, such lesions are seen after staphylococcal pneumonias (pneumatocele). They may disappear spontaneously. Rarely, surgical extirpation is required. There is no recurrence.

     In the adult, isolated bullae may be found as the sequelae to a tuberculous process treated with antimicrobial drugs, notably isoniazid.
   - *B) Multicentric:* This type of bullous disease may be stationary, complicated by infection or pneumothorax, or may go on to progressive bullous emphysema (vanishing lung) with compression of healthy lung tissue. Treatment is symptomatic. Surgical removal of large bullae may relieve re-

spiratory insufficiency. Recurrence is possible.

Summary

Two families are reported in which various members were affected by pulmonary emphysema. Observations of this sort permit the inference that genetic factors play a part in the pathogenesis of this condition. Family surveys of patients with pulmonary emphysema, especially when the usual causal factors are not evident or when the condition appears at an early age, may uncover further instances of this kind.

Sumario

Enfisema Familiar

Preséntanse los casos de dos familias, varios miembros de las cuales estaban afectados por enfisema pulmonar Las observaciones de este género permiten la inferencia de que factores genéticos desempeñan un papel en la patogenia de esta dolencia. Estudios de las familias de los enfermos de enfisema pulmonar, en particular cuando no son evidentes los habituales factores etiológicos o cuando la afección aparece a una edad temprana, tal vez descubran nuevos casos de esta especie.

Resume

Emphysème familial

Les auteurs rapportent deux familles chez lesquelles différents membres furent affectés par de l'emphysème pulmonaire. Des observations de cette sorte permettent de penser que des facteurs génétiques jouent un rôle dans la pathogènie de cette condition. Une étude familiale des malades ayant de l'emphysème pulmonaire, spécialement quand les facteurs habituels de cause ne sont pas evidents, ou lorsque cette condition survient à un age précoce, peut découvrir d'autres exemples de cette sorte.

Acknowledgments

The data on family *B* were provided by Drs. Robert G. Bloch, W. R. Barclay, and Benjamin Burrows. Dr. Bloch observed patients *P.C.*, *R.C.*, and *G.D.* when on the chest service of the University of Chicago Clinics. The follow-up data and information on other members of this family were provided by Dr. W. R. Barclay at that institution.

The writers thank the aforementioned physicians for their kindness in permitting the use of this information. They wish to express their indebtedness to Drs. Robert G. Bloch and Eli H. Rubin of the Pulmonary Division of the Montefiore Hospital for their helpful advice and kind encouragement.

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THE OCCURRENCE OF AZOTEMIA IN PNEUMOCOCCAL PNEUMONIA[1]

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INTRODUCTION

The advent of antimicrobial therapy, although greatly simplifying the management of patients with pneumococcal pneumonia, has not decreased the frequency of the disease (1, 2). Recently, the writers have been impressed by the frequency with which azotemia seemed to occur in patients with uncomplicated pneumococcal pneumonia. Since this finding has been responsible for prolonging hospitalization in many patients, it seemed important to review this association.

In the texts dealing with lobar pneumonia, azotemia is mentioned only by Bullowa (3–8) as a feature of the disease. However, the occurrence of azotemia in the course of uncomplicated pneumococcal (lobar) pneumonia received considerable attention prior to 1930 (9–26) (table 1). In several of the early studies, it was concluded that renal function was increased despite the occurrence of azotemia (20, 24, 25). Such conclusions were based upon estimations of urea clearance which has definite limitations under the circumstances of study. These reports have subsequently served to obscure the frequency and importance of azotemia in pneumococcal pneumonia. Azotemia has been observed occasionally in association with a variety of acute, infectious processes; however, the frequency of occurrence in patients with pneumococcal pneumonia seemed greater than in those with other serious infections (9, 12, 13, 15, 16).

The present study is being reported both to re-emphasize the frequent occurrence of significant azotemia during the course of otherwise uncomplicated treated pneumococcal pneumonia and to characterize the clinical features of such patients.

MATERIALS AND METHODS

The hospital records of all patients with the diagnosis of pneumococcal pneumonia who had been seen and treated on the medical wards of Parkland Memorial Hospital during the winter of 1957–1958 were reviewed. The patients were divided into two groups: one consisted of a group of 44 patients with clinical features typical of pneumococcal pneumonia and in whom the bacteriologic diagnosis was established by isolation of *Diplococcus pneumoniae* from either blood and/or sputum culture; the other consisted of 24 patients with histories typical of pneumococcal pneumonia, lobar consolidation, and leukocytosis, but in whom definitive bacteriologic diagnoses were not made.

The following information was sought from each record: age, sex, duration of symptoms, history of renal disease, history of alcoholism, physical state and nourishment, extent of pulmonary involvement (by roentgenogram), pertinent physical findings, blood chemical values (blood urea nitrogen, bilirubin, creatinine, and chloride), peripheral leukocyte counts, mode of treatment, and course of the illness.

All biochemical determinations were done in the routine clinical hospital biochemistry laboratory. An auto-analyzer, employing the diacetyl monoxime reaction, was used for determination of blood urea nitrogen, and the Jaffe alkaline picrate reaction for the determination of serum creatinine.

Conclusions of this study were based upon the observations made in the group of 44 patients in whom a definitive bacteriologic diagnosis was established. The other group, in which diagnoses were solely on clinical grounds, is presented to corroborate the observations in the first group.

The significance of differences between various observations was tested by the method of chi-square using 2×2 contingency tables and applying Yates correction for discontinuity in small series.

RESULTS

The frequency with which azotemia occurs in association with pneumococcal pneumonia is recorded in table 2. A blood urea nitrogen concentration in excess of 25 mg. per 100 ml. was arbitrarily chosen as a dividing point, as this concentration is unequivocally increased. Twenty-one of the patients had concentrations in excess of this value (48 per cent). Azotemia was observed frequently in both patients with bacteremia in association with their pneumonia (77 per cent) and patients with sterile blood cultures (42 per cent). While azotemia appeared

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TABLE 1

ASSOCIATION OF AZOTEMIA* AND LOBAR PNEUMONIA

Author	Reference Number	Number of Patients	Azotemia		Comments
			Number	Per Cent	
Tileston, 1914	9	14	7	50	
Foster, 1915	10	—	—	—	"Increase in nonprotein nitrogen exceptional"
Rackemann, 1916	11	9	7	78	
Schwartz, 1916	12	36	33	92	
Bookman, 1917	13	4	1	25	
Lewis, 1917	14	8	0	0	3 cases > 20 mg. per 100 ml.
Frothingham, 1918	15	10	3	30	
Cohn, 1922	16	55	48	87	
Killian, 1922	17	50	Occurred		Various types of pneumonia
Wilcox, 1923	18	47	2	4	Children, creatinine < 2.0 mg. per 100 ml.
Haden, 1925	19	6	4	66	Creatinine < 2.0 mg. per 100 ml.
McIntosh, 1926	20	13	7	54	
Peters, 1926	21	5	2	40	1 case of hemolytic streptococcosis and tuberculosis
Sunderman, 1926	22	19	8	50	1 case of hemolytic streptococcosis and pneumococcal pneumonia
Haden, 1927	23	7	4	57	
Goldring, 1931	24	13	2	15	
Farr, 1937	25	27	4	15	
Peters, 1946	26	19	11	58	Nonprotein nitrogen > 40 mg. per 100 ml.
Total		292	143	49	

* Azotemia empirically chosen as concentrations of blood urea nitrogen in excess of 25 mg. per 100 ml. or nonprotein nitrogen in excess of 50 mg. per 100 ml.

more frequent in patients with bacteremia, in this small series such a difference is not significant ($P < 0.20$).

The relationship between various other clinical features and the occurrence of azotemia is listed in table 3. Azotemia occurred with equal frequency in both young and elderly patients and in both sexes. Likewise, there was no correlation between the occurrence of azotemia and the duration of illness prior to hospital admission. A history of renal disease or antecedent elevation of the blood urea nitrogen was obtained in only 3 patients. All of these patients had an increase in the degree of azotemia, and 2 of the 3 patients died. Serious infection as manifested by features other than bacteremia, specifically extensive pulmonary involvement or jaundice, also did not correlate closely with the occurrence of azotemia. However, only one patient had hypotension or leukopenia.

Hypochloremia is a common feature in pneumococcal pneumonia (19, 21–23). Concentrations of serum chlorides of less than 100 mEq/L. were observed in 71 per cent of the patients in whom determinations were performed (table 3). While the over-all frequency of hypochloremia in patients without azotemia (73 per cent) was comparable to that in patients with azotemia (70 per cent), marked hypochloremia (<90 mEq/L.) occurred only in patients with associated azotemia. Determinations of the concentrations of serum sodium were not routinely performed;

TABLE 2

BLOOD UREA NITROGEN CONCENTRATIONS IN PATIENTS WITH PNEUMOCOCCAL PNEUMONIA
(Number of Cases)

Blood Culture	Number of Patients	Blood Urea Nitrogen (mg. per 100 ml.)						Per Cent of Blood Urea Nitrogen > 25 mg. per 100 ml.
		<15	15–25	26–40	41–60	61–100	>100	
Positive	13	0	3	3	2	4	1	77
Negative	19	2	9	1	1	4	2	42
Not done	12	6	3	3	0	0	0	25
Total	44	8	15	7	3	8	3	48

hence, associated occurrence of hyponatremia could not be ascertained in these studies.

Serum creatinine concentrations were determined in 4 of the 21 patients with azotemia (table 4). The single patient with a markedly elevated concentration of creatinine in the serum failed to survive.

Follow-up determination of the blood urea nitrogen was obtained in 17 of the 21 patients with elevated concentrations (figure 1). The azotemia disappeared (blood urea nitrogen, 15 mg. per 100 ml. or less) within fourteen days after therapy in 6 patients (29 per cent). Of the remaining 11 patients, 8 demonstrated progressive decreases in their blood urea nitrogen values which returned to normal or near normal concentrations during the period of observation (sixteen to twenty-six days following admission). Three subjects, 2 of whom had histories of preexisting renal disease, died while being treated for pneumonia. These patients showed rapid increases in their azotemia and associated hyperkalemia (serum potassium 7.7 mEq. per liter, 7.4 mEq. per liter, and 7.1 mEq. per liter). The other patients did not show hyperkalemia.

In the group of 24 patients with clinical diagnoses of pneumococcal pneumonia, the concentration of blood urea nitrogen exceeded 25 mg. per 100 ml. in 9 patients (37 per cent). The observations made in these patients correspond with those in the group with bacteriologically proved pneumococcal pneumonia.

To determine whether azotemia was characteristic of pneumococcal infections in general, the records of 12 adult patients with pneumococcal meningitis without pneumonia were reviewed. None of these patients had blood urea nitrogen concentrations exceeding 25 mg. per 100 ml.

TABLE 3

RELATIONSHIP OF VARIOUS CLINICAL FEATURES TO THE OCCURRENCE OF AZOTEMIA

Clinical Feature	Total Cases	Blood Urea Nitrogen Concentration	
		>25 Mg. per 100 Ml.	>25 Mg. per 100 Ml.
Age (years)			
<20	3	—	3
20–40	13	5	8
40–60	10	7	3
>60	18	9	9
Sex			
Male	26	14	12
Female	18	7	11
Duration of symptoms (days)			
<2	7	2	5
2–6	19	11	8
7–14	9	5	4
>14	9	3	6
History of renal disease			
Yes	3	3	—
No	32	12	20
Extent of pulmonary involvement			
1 lobe	26	11	15
2 lobes	9	4	5
>2 lobes	1	1	—
Bronchopneumonia	8	5	3
Serum bilirubin			
Normal	8	4	4
Elevated (>2.0)	9	5	4
Leukocytosis >15,000			
Yes	20	11	9
No	24	10	14
Serum chloride			
>100 mEq/L.	10	6	4
90–99 mEq/L.	19	8	11
<90 mEq/L.	6	6	0

TABLE 4

RELATIONSHIP BETWEEN BLOOD UREA NITROGEN AND SERUM CREATININE CONCENTRATIONS

Patient Number	Age	Sex	History of Renal Disease	Initial Blood Urea Nitrogen (mg. per 100 ml.)	Initial Creatinine (mg. per 100 ml.)	Outcome
163992	55	M	No	65	1.2	Survived
106349	70	F	No	41	1.5	Survived
W19877	29	F	No	31	2.0	Survived
W14013	47	F	Yes*	130	7.21	Died

* Probable renal calculus three years earlier.

DISCUSSION

The present study substantiates earlier observations that azotemia is a common occurrence in the course of uncomplicated pneumococcal pneumonia. Likewise, the frequency is approxi-

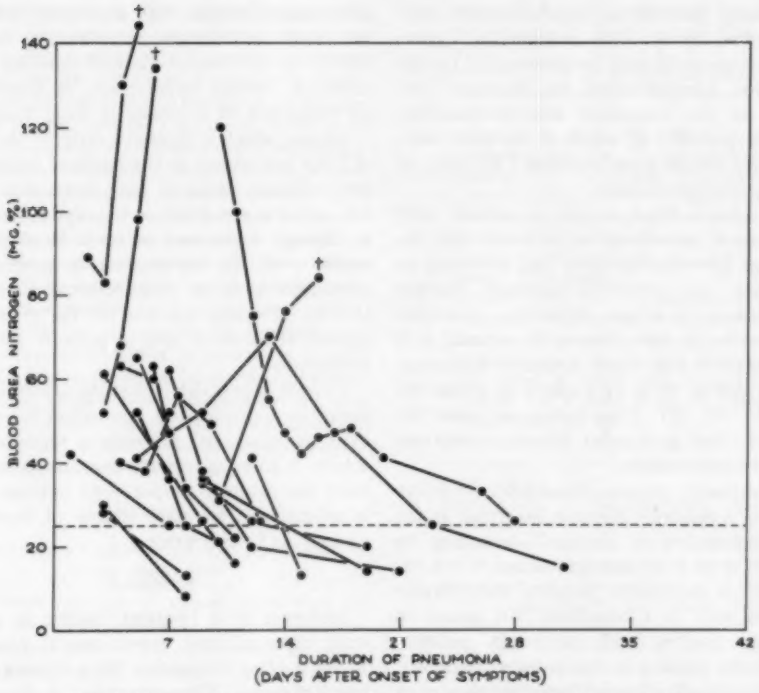

FIG. 1. Course of azotemia in patients with pneumococcal pneumonia.

mately the same as that compiled from reports published prior to 1930, i.e., 48 per cent in the present series as compared with a composite of 49 per cent in earlier reports (*see* table 1). There was no definite correlation between azotemia and other manifestations of overwhelming infection, such as bacteremia, hypotension, multiple lobe involvement, leukopenia, or jaundice. Cohn (16) reported no correlation between the extent of pulmonary involvement, temperature or duration of symptoms, and the occurrence of azotemia.

Earlier workers failed to agree on the prognostic significance of azotemia. Some regarded it as a grave prognostic feature (12, 17), whereas others presented evidence indicating that azotemia was not associated with an altered course (9, 16). The present writers' observations support the view that azotemia is not a grave prognostic finding unless pneumococcal pneumonia occurs in patients with coexistent renal disease. Although there are too few patients in this series to ascertain accurately the gravity of pneumococcal pneumonia in patients with underlying renal disease, it is of note that 2 of the 3 patients with previous renal disease died. In the report of Sunderman, Austin, and Camac (22), only one patient had underlying renal disease as measured by an increase in serum creatinine, and this patient, a 46-year-old male, died. The 2 patients with stable chronic renal insufficiency, and a third with probable antecedent renal insufficiency, who developed pneumococcal pneumonia and died, had acute hyperkalemia with concentrations of serum potassium of 7.7, 7.4, and 7.1 mEq. per liter terminally.

An electrocardiogram was obtained in one patient (No. 165451) late in her course which showed peaking of T waves in precordial leads V-3 and V-4. These observations suggest that pneumococcal pneumonia may be a very serious illness in the face of stable chronic renal insufficiency. The catabolic response which occurs with acute infections results in excessive loss of nitrogen and potassium as well as of other cellular constituents. In the face of underlying serious renal

insufficiency, potassium concentrations may reach lethal levels. This endogenous hyperkalemia occasionally may be accentuated by the enthusiastic administration of exogenous potassium, as the potassium salt of penicillin. Potassium penicillin G, which is the most commonly used dosage form, contains 1.68 mEq. of potassium per million units.

The azotemia which occurs in patients with pneumococcal pneumonia is primarily the result of an increased nitrogen load delivered to the kidney, i.e., pre-renal azotemia. Normal concentrations of serum chromogen creatinine were observed in the presence of azotemia in 3 of 4 patients in this series. A similar dichotomy was recorded in 10 of 11 patients in earlier reports (18, 19, 22). This finding supports the hypothesis that glomerular filtration rates are not severely decreased.

The increased protein breakdown probably represents a catabolic response described as the "toxic destruction of protein." According to Peters (27), such protein destruction is not encountered in debilitated patients with chronic infections such as tuberculosis, but occurs in previously healthy well nourished patients. Many of the patients in this series were elderly, poorly nourished subjects; however, they may not have been debilitated to an extent necessary to mitigate the catabolic response to infection. The mechanism of protein wastage which accompanies acute infections has not been definitively established. An acute infection such as meningococcal meningitis usually is associated with a marked negative nitrogen balance; moreover, nitrogen balance often was not restored by large increases in nitrogen intake (28). Such metabolic alterations resemble the effects of excessive amounts of adrenal cortical hormones, and adrenocortical stimulation has been shown to follow a stress such as infection. Engel (29) has summarized observations which suggest that the intervention of the adrenal cortex may represent a "permissive" rather than direct effect. Adrenalectomized animals maintained on a constant dose of hormone can respond to stress in a normal manner with respect to most morphologic and metabolic phenomena, and the negative nitrogen balance which follows stress occurs significantly sooner than that induced by corticotropin or adrenocortical hormone.

In addition to a major pre-renal component, moderate decreases in renal blood flow and glomerular filtration rate may occur which do not result in retention of creatinine, but contribute to the magnitude and duration of the azotemia. Anoxia, hypotension, or fever would all contribute to decreases in renal blood flow.

Anoxia, which is frequent early in the course of lobar pneumonia as the result of perfusion of nonventilated areas of lung, may also play a role either as a stimulus to the catabolic response or through a decrease in renal blood flow. As anoxia would be less common in pneumococcal meningitis than in pneumococcal pneumonia, this factor might account for the greater frequency of azotemia seen in patients with lobar pneumonia.

Thus, it is postulated that the azotemia which occurs in approximately one-half of the patients with pneumococcal pneumonia represents primarily a form of pre-renal azotemia resulting from the catabolic response to infection which is accentuated by some degree of decrease in glomerular filtration rate.

Summary

Azotemia is a frequent finding in patients with uncomplicated pneumococcal pneumonia which is often responsible for a prolongation of hospitalization. Concentrations of blood urea nitrogen greater than 25 mg. per 100 ml. were observed in 48 per cent of 44 patients with bacteriologically proved pneumococcal pneumonia and in 37 per cent of a group of 24 patients with clinical pneumococcal pneumonia. This phenomenon represents primarily a form of pre-renal azotemia and is not a grave prognostic finding, unless the pneumococcal pneumonia occurs in patients with antecedent renal insufficiency. In patients wtih antecedent renal insufficiency, lethal hyperkalemia may be a consequence of the catabolic response evoked by the infection. Determination of the serum creatinine concentration is helpful in the differentiation of primary pre-renal azotemia with pneumococcal pneumonia (in which the concentration is normal) from underlying renal insufficiency with associated pneumococcal pneumonia (in which the concentration is elevated).

Sumario

La Ocurrencia de Azoemia en la Neumonía Neumocócica

La azoemia es un hallazgo frecuente en los enfermos de neumonía neumocócica sin complica-

ciones, y es a menudo la causa de una prolongación de la hospitalización. Observáronse concentraciones de nitrógeno de urea sanguínea superiores a 25 mgs. por 100 mls. en 48 por ciento de 44 enfermos que tenían neumonía neumocócica comprobada bacteriológicamente y en 37 por ciento de un grupo de 24 enfermos que tenían neumonía neumocócica clínica. Representa este fenómeno primordialmente una forma de azoemia prerrenal y no constituye un signo pronóstico grave, a menos que la neumonía neumocócica recaiga en sujetos con insuficiencia renal antecedente. En los enfermos con esta insuficiencia, una hiperpotasemia letal puede ser la consecuencia de la respuesta catabólica provocada por la infección. La determinación de la concentración sérica de creatinina resulta útil en la diferenciación de la azoemia prerrenal primaria con neumonía neumocócica (en la que la concentración es normal) de la insuficiencia renal subyacente con neumonía neumocócica asociada (en la que se eleva la concentración).

Resume

L'apparition d'azotémie dans la pneumonie à pneumoccoques

L'azotémie est trouvée fréquemment chez les malades avec une pneumonie à pneumoccoques sans complications, ce qui est souvent la raison d'une hospitalisation prolongée. Les auteurs observèrent des concentrations d'azote uréique sanguin supérieures à 25 mg pour 100 ml, chez 48% de 44 malades ayant fait la preuve bactériologique d'une pneumonie à pneumoccoques, et chez 37% d'un groupe de 24 malades avec une pneumonie à pneumoccoques cliniquement. Ce phénomène représente en premier lieu une forme d'azotémie pré-rénale et n'est pas une donnée de pronostic grave à moins que la pneumonie à pneumoccoques ne survienne chez des malades avec des antécédents d'insuffisance rénale. Chez les malades ayant des antécédents d'insuffisance rénale, une hyperkaliémie mortelle peut être une conséquence de la réponse catabolique entrainée par l'infection. La détermination de la concentration sérique de créatinine est utile pour différencier une azotémie pré-rénale primaire avec pneumonie à pneumoccoques (dans laquelle la concentration est normale) d'une insuffisance rénale sous-jacents associée à une pneumonie à pneumoccoques (dans laquelle la concentration est élevée).

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ISONIAZID PROPHYLAXIS OF TUBERCULOUS INFECTION IN MICE[1-3]

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INTRODUCTION

In the preventive measures against tuberculosis, as in other infectious diseases, the first objective must be to prevent infection by diminishing exposure of individuals to *M. tuberculosis* and, when infection has been established, as shown by a positive tuberculin reaction, another method must be tried to prevent such infection from developing into tuberculous disease.

From July, 1957, to June, 1958, a survey was made on randomly selected subjects to determine the prevalence of pulmonary tuberculosis in Taiwan. This survey showed the over-all prevalence of pulmonary tuberculosis to be about 2 per cent (200,000) as judged roentgenographically, and 0.5 per cent (50,000) as determined bacteriologically (1). Recently, 13,000 to 15,000 infectious cases were estimated to be present (2).

It is obvious, therefore, that tuberculosis cannot be prevented solely by relying on the isolation of these cases. The attempt to control the disease by vaccination has proved to have only limited success. A series of laboratory studies have been reported on the prevention of experimental tuberculosis in laboratory animals by the use of isoniazid (3). Several of these studies were reported by Ferebee, Palmer, and Hopwood (4, 5). The results of these experiments indicated that the optimal prophylactic dose should lie somewhere between 1 and 5 mg. per kg. per day and that guinea pigs can be protected against massive inoculation of virulent *M. tuberculosis* by isoniazid prophylaxis.

The present series consisted of experiments with small doses of isoniazid in mice. The experiments were designed to determine the preventive effect of isoniazid in experimental tuberculosis in mice, and to see whether isoniazid by itself gives protection or whether it merely suppresses the infection and requires the further participation of an immune response to effect lasting protection.

MATERIALS AND METHODS

Strain: The stock laboratory strain of *M. tuberculosis* H37Rv was obtained through the courtesy of Prof. L. C. Yen from the standard culture collection maintained by the Shihlin Provincial Serum Institute, Taipei, Taiwan.

Mice: The susceptible strain of mice, C57, was also kindly suplied by the same Institute.

Drug: Isonicotinic acid hydrazide (isoniazid), USP, XV, BPC, from Italy, was purchased locally.

Study plan: Six identical experiments were performed. Each experiment required 600 mice which were kept in thirty cages (20 mice of the same sex per cage) and divided into six groups. The entire study involved a total of 3,600 mice, 600 mice being used for each group. The treatment schedule is presented in table 1.

Determination of water consumption: Because the isoniazid was given to the mice in their drinking water, a relatively dry diet was used so as to force the animals to obtain fluid from the water. Before the experiments started, it was necessary to determine the average water consumption of mice per kg. of body weight per day. For measurement purposes the water was provided so as to avoid spilling, thus ensuring that the amount of water lost would approximate the amount drunk. The drinking water of the animals was changed routinely every day, but the amount consumed was measured for only one twenty-four-hour period each week for several weeks. The average maximal amount of water for a normal mouse during a period of twelve weeks approximated 380 ml. per kg. of total body weight per day. The mice drank a somewhat smaller amount of water containing isoniazid. During the first four and one-half weeks after receiving isoniazid, mice in the experimental groups drank an average of 100 ml. per kg. of total body weight per day. To this amount, 3 mg. of isoniazid per kg. of body weight were added.

Challenge: The mice of *groups 1–3* were challenged intraperitoneally with 1.5 mg. wet weight (LD_{90}) of virulent *M. tuberculosis* H37Rv after receiving isoniazid for four and one-half weeks.

After this time, all of the mice in *groups 2* and *5* continued to receive the usual amount of isoniazid for ten more weeks. During this period a systemic observation was made of all groups, the

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[2] This paper was presented at the biennial meeting of the Chinese Medical Association, Taipei, May 8, 1960.

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TABLE 1

STUDY PLAN AND TOTAL NUMBER OF MICE IN THE EXPERIMENTAL GROUPS

Group	Isoniazid Started	Challenge at 4½ Weeks	Isoniazid Continued	Challenge at 10 Weeks
	Duration of Observation			
	4½ Weeks	10 Weeks		14 Weeks
1	3 mg./kg./day (600)	H37Rv (549)	None	H37Rv (393)
2	3 mg./kg./day (600)	H37Rv (554)	3 mg./kg./day	H37Rv (423)
3	None (600)	H37Rv (548)	None	None (313)
4	3 mg./kg./day (600)	None (547)	None	H37Rv (464)
5	3 mg./kg./day (600)	None (555)	3 mg./kg./day	H37Rv (478)
6	None (600)	None (547)	None	H37Rv (467)

TABLE 2

THE MORTALITY RATE OF MICE BEFORE CHALLENGE

Group	Isoniazid	Number of Mice	Total Body Weight	Mice Dead before Challenge:	
				Number	Mortality
			gm.		*per cent*
1	Fed	600	12,855	51	8.5
2	Fed	600	13,470	46	7.67
3	Not fed	600	13,375	52	8.66
4	Fed	600	12,856	53	8.83
5	Fed	600	13,870	45	7.50
6	Not fed	600	13,075	53	8.83

water consumption per cage was measured, and the mice were weighed weekly. The dead animals were noted daily, and autopsies were performed. The causes of death, especially if other than tuberculous infection, were carefully analyzed by use of direct microscopy with acid-fast stain. Ten weeks after the first challenge, i.e., fourteen and one-half weeks after the beginning of the experiment, *group 3* remained as the infection control group, and the other five groups were all challenged by the same dosage and method used in the first challenge. This was done in order to investigate the degree of protection produced solely by isoniazid and that given by isoniazid combined with partial immunity ensuing from experimental tuberculous infection.

Histopathologic study: The mice were kept under observation for fourteen weeks after the second challenge. The survivors of each group were then killed, autopsied, and some of the specimens obtained at autopsy (including the lungs, spleens, livers, and kidneys of each group) were selected at random, fixed in 10 per cent formalin, and prepared for pathologic study. At the end of the experiment, the lung specimens obtained from the mice of *groups 1, 2, 4,* and *5* (that had received isoniazid for various periods) were used for the re-isolation of *M. tuberculosis*. The tubercle bacilli thus isolated were tested for susceptibility to isoniazid by routine methods (6, 7).

RESULTS

During the first four and one-half weeks of isoniazid administration before the first challenge, the mortality rate of the mice in each group was almost equal. It was 8.125 per cent in the isoniazid group and 8.75 per cent in the group that received no drug (table 2).

Ten weeks after the first challenge, the mice of the infected control group (*group 3*) died rapidly of tuberculous infection and had a high mortality rate (36.7 per cent). The mice of experimental *group 1* and, especially, *group 2*, showed a lower mortality rate (16.25 per cent average) than the control group (table 3).

The mortality rates of the mice in each group fourteen weeks after the second challenge are listed in table 4. While the previously and newly infected control groups (*groups 3* and *6*) showed higher mortality rates of 53.9 to 68.3 per cent, the experimental groups showed rates of 17.3 to 25.2 per cent.

Among the experimental groups, the mice of *group 2* showed the lowest mortality rate. This may be due to the fact that these mice were treated with isoniazid after the first challenge and, therefore, partial immunity against tuberculous infection had been developing during the course of treatment. The mice of *group 1* that did not have isoniazid treatment after the first challenge might have had weaker protection and thus developed progressive disease.

The general condition of the mice in the control groups (*groups 3* and *6*), as evidenced by loss of body weight, became worse day by day in

TABLE 3

MORTALITY OF MICE 10 WEEKS AFTER FIRST CHALLENGE

Group	Challenge	Number of Mice	Total Body Weight	Isoniazid	Number of Mice that Died:		Mortality from Tuberculous Infection
					From Other Causes	From Tuberculous Infection	
			gm.				*per cent*
1	Challenged	549	12,640	Discontinued	59	97	17.7
2	Challenged	554	13,460	Continued	49	82	14.8
3	Challenged	548	13,275	None	34	201	36.7
4	Not challenged	547	13,160	Discontinued	83	—	—
5	Not challenged	555	13,680	Continued	77	—	—
6	Not challenged	547	13,065	None	80	—	—

TABLE 4

MORTALITY OF MICE 14 WEEKS AFTER SECOND CHALLENGE

Group	Challenge	Number of Mice	Total Body Weight	Number of Mice That Died:		Mortality from Tuberculous Infection
				From Other Causes	From Tuberculous Infection	
			gm.			*per cent*
1	Rechallenged	393	9,557	15	109	25.2
2	Rechallenged	423	11,156	22	73	17.3
3	Not challenged	313	6,748	15	194	68.3
4	Challenged	464	11,640	23	90	19.3
5	Challenged	478	11,724	29	90	18.8
6	Challenged	467	10,183	17	252	53.9

the course of the experiment as compared with the other experimental groups (*groups 1, 2, 4,* and *5*) (table 5).

Pathologic findings: A total of 61 sets of organs was examined. Paraffin sections were made from fixed tissue and stained with hematoxylin and eosin. Special stains such as the acid-fast and the amyloid stains were also used. The histologic changes in the organs of all groups were fundamentally similar and differed only in extent. The description deals first with the organs singly in order of general severity of the changes. The differences in the pathologic changes found in the six groups of mice are presented in table 6.

Lungs: The microscopic findings in the lungs (8), for the convenience of description, are divided as follows: (*1*) normal: no abnormalities were observed; (*2*) minimal histologic change: there was only slight infiltration of mononuclear cells in the alveolar walls and peribronchial tissue; (*3*) single caseous necrosis: this represented a solitary lesion of caseation; (*4*) bronchopneumonia: this was lobular in pattern; the infiltrate was composed of large mononuclear cells and lymphocytes; neutrophilic leukocytes were also found, especially in the necrotic areas; (*5*) confluent bronchopneumo-

TABLE 5

COMPARISON OF AVERAGE BODY WEIGHT (in Grams) OF MICE IN THE EXPERIMENTAL GROUPS

Group	At the Start of the Experiment	10 Weeks after Challenge	14 Weeks after Challenge	Remarks
1	21.4	24.4	24.8	Received 3 mg. of isoniazid per kg. per day for 4½ weeks before first challenge
2	22.4	24.0	27.9	Received 3 mg. of isoniazid per kg. per day for 4½ weeks before first challenge and 10 weeks after first challenge
3	22.2	21.5	20.1	Control animals designated to check the virulence of the first challenge
4	21.4	22.9	24.3	Treated the same as *group 1*
5	23.1	24.5	25.6	Received the same dose of isoniazid for 14½ weeks before challenge
6	21.7	23.7	22.8	Control animals designated to check the virulence of superinfection

TABLE 6

HISTOPATHOLOGIC CHANGES IN THE MICE IN EACH EXPERIMENTAL GROUP

Mouse Organ	Pathologic Changes*	Experimental Group					
		1(5)†	2(18)	3(10)	4(5)	5(18)	6(13)
Lungs	Normal		10(55%)			3(11%)	
	Minimal histologic change				1(20%)	11(41%)	
	Single caseous necrosis	1(10%)				3(11%)	
	Bronchopneumonia	4(80%)	8(45%)	1(10%)	3(60%)	7(25.6%)	1(7.7%)
	Confluented bronchopneumonia:						
	Advanced	1(10%)		8(80%)	1(20%)	2(7.4%)	10(77%)
	Moderate					1(4%)	2(15.3%)
	Caseous pneumonia			1(10%)			
Spleen	Caseous necrosis:						
	Extensive			1			1
	Moderate					4	5
	Amyloidosis:						
	Advanced		8	2		3	4
	Moderate		8	1		20	9
	Reticuloendothelial activity:						
	Advanced	3	8	5	4	7	4
	Moderate	2	7	2	1	19	9
Liver	Caseous necrosis:						
	Advanced	3	1	2		6	2
	Moderate	1	2	4		1	4
	Amyloidosis:						
	Advanced		9				9
	Moderate	1	4		1	22	4
	Reticuloendothelial activity:						
	Advanced		7	2	4	8	8
	Moderate		8	2		19	5

* *See* text for full explanation of changes.

† Numbers in parentheses indicate number of mice examined.

nia: the lesions were enlarged and merged into each other; a large proportion of the lung parenchyma was affected; in the severe cases, there was almost no intervening normal tissue to be found; (*6*) caseous pneumonia: in this variety the inflammatory exudate had more or less converted into caseous material; in the severely involved areas the alveolar walls were destroyed.

Spleen: Three types of lesions were seen: (*1*) caseous necrosis: the extent of necrosis was variable; some lesions were microscopic and others were extensive and irregular in shape; (*2*) amyloidosis: this was diffused in type but variable in degree; the amyloid deposits in the sinusoidal septum were seen as masses or columns of hyaline material; in severe cases large areas of splenic pulp were obliterated; (*3*) reticuloendothelial activity: this consisted of diffuse infiltration of large mononuclear cells and giant cells in the pulp cords and sinuses; the giant cells were of the foreign-body giant-cell type probably related to the presence of amyloid.

Liver: The changes in this organ closely resembled those in the spleen. The caseous necrosis was sometimes found to be contiguous with a caseous lymph node. In such instances, the liver, stomach, and spleen adhered to one another. Atrophy of hepatic cords was conspicuous when deposition of amyloid was great. This occurred mainly in the central zone of the lobule. In the severely affected cases, the infiltration was diffused.

Kidney. Not remarkable.

At the end of the experiment, 18 strains of *M. tuberculosis* were re-isolated. Their susceptibility to isoniazid was tested, and no isoniazid-resistant strains were found.

DISCUSSION

In recent years, in the course of studies on the therapeutic effect of isoniazid, several workers (9–14) have made observations on the prophylactic effect of this drug. The general methods employed to determine whether isoniazid could be used to prevent experimental tuberculosis in guinea pigs have been described in previous reports (4, 5). According to the present data from studies in mice, a daily dose of 3 mg. of isoniazid per kg., if started and continued for

four and one-half weeks before the challenge, appeared to lower the mortality from tuberculous infection. The mortality of the isoniazid groups ranged from 14.8 to 17.7 per cent, while that of the control group was 36.7 per cent. Fourteen weeks after a second challenge, the previously and newly infected control animals showed higher mortality rates of 53.9 to 68.3 per cent, while the prophylactic groups had lower mortality rates of 17.3 to 25.2 per cent.

The animals of *group 2*, which had been given isoniazid continously for ten weeks after the first challenge, showed the lowest mortality.

Isoniazid treatment does not regularly eradicate all of the tubercle bacilli of a large population (15, 16), as shown in the Ferebee-Palmer experiment mentioned above. If the guinea pigs get a fairly large dose of isoniazid during the first challenge, their protection against a rechallenge seems to be less than if they had a smaller but still effective dose. This would also appear to indicate that the protection afforded by isoniazid does not depend on the eradication of all bacteria after massive infection but, rather, on the rapid reduction of the size of the challenge population of tubercle bacilli or the inhibition of their migration. Thus, the danger of dissemination of disease from the original foci is minimized. At the same time, the chemoprophylaxis allows enough organisms to remain alive in the lesions so as to build up a degree of acquired immunity. It is also possible that immunity developed with such a large challenge dose at the time of the first challenge. Observations on this point have been presented in various papers (17–20) by Dubos and his associates.

Postmortem examinations of gross and histopathologic findings in the mice killed after twenty-four weeks of observation in the present experiments corroborated the evidence of prophylaxis provided by the lower mortality rates in isoniazid-treated animals. The infected control mice died rapidly, as had been expected, and 77 to 80 per cent showed manifestations of confluent bronchopneumonia (*see* table 6). The above findings, therefore, indicated that isoniazid was effective in preventing the spread of infection from the primary foci. These results in mice are in agreement with the clinical findings of Ferebee and her associates (21), who discovered that isoniazid administration to children with asymptomatic primary tuberculosis decreased the incidence of serious extrapulmonary tuberculous complications, such as miliary tuberculosis and tuberculous meningitis. It is reasonable to suppose that isoniazid might not completely inhibit the migration of bacteria from the primary focus, but it should effectively suppress any multiplication of bacteria that were deposited at other sites. It is probable, therefore, that in this experiment bacteria in small numbers did reach the lungs and other organs of the treated animals, but failed to multiply there.

Summary

Isoniazid in a dosage of 3 mg. per kg. per day for four and one-half weeks exerted a prophylactic effect in experimentally infected tuberculous mice. The resistance to infection was demonstrable even after the drug had been discontinued. During the period of observation, the mortality in the isoniazid groups was 14.8 to 17.7 per cent, while that in the control group was 36.7 per cent. Fourteen weeks after the second challenge, the previously and newly infected control animals showed higher mortality rates of 53.9 to 68.3 per cent. By contrast, in the prophylactic groups the mortality rates were 17.3 to 25.2 per cent.

The infected mice that received the same doses of isoniazid for four and one-half weeks before challenge and ten weeks thereafter acquired some degree of immunity against rechallenge. Fourteen weeks after rechallenge, in more than half of the prophylactic group the lungs were normal. The histopathologic changes in all organs (lungs, liver, spleen, and kidney) were considerably less in the mice that received isoniazid than among the control animals. At the end of the experiment, no isoniazid-resistant strains of *M. tuberculosis* were isolated from any of the animals.

Sumario

Profilaxis con Isoniacida de la Infección Tuberculosa en los Ratones

La isoniacida, a dosis de 3 mg. por kg. de peso al día durante cuatro semanas y media, ejerció efecto profiláctico en los ratones infectados experimentalmente con tuberculosis. La resistencia a la infección era observable aun después de discontinuar la droga. Durante el período de observación, la mortalidad en los grupos tratados con isoniacida

fué de 14.8 17.7 por ciento mientras que en el grupo testigo fué de 36.7 por ciento. A las catorce semanas de la segunda provocación, los testigos primitiva y recientemente infectados revelaron tasas de mortalidad más altas, de 53.9 a 68.3 por ciento. En contraposición, en los grupos profilácticos las tasas de mortalidad dueron de 17.3 a 25.2 por ciento.

Los ratones infectados que recibieron las mismas dosis de isoniacida durante cuatro semanas y media antes de la provocación y diez semanas después adquirieron alguna inmunidad contra la re-provocación. A las catorce semanas de ésta, en más de la mitad del grupo profiláctico los pulmones eran normales. Las alteraciones histopatológicas en todos los órganos (pulmones, hígado, bazo y riñón) eran considerablemente menores en los ratones que recibieron isoniacida que entre los animales testigos. Al final del experimento, no se aislaron cepas isoniacido-resistentes del *M. tuberculosis* de ninguno de los animales.

Resume

Prophylaxie de l'infection tuberculeuse chez des souris grace à l'isoniazide

A une dose journaliére de 3 mg par kg, pendant quatre semaines et demi, l'isoniazide exerce un effet prophylactique chez des souris tuberculeuses infectées expérimentalement. On put démontrer la résistance à l'infection, même après qu'on ait cessé le médicament. Pendant cette période d'observation, la mortalité chez les groupes à l'isoniazide fut de 14,8 à 17,7% tandis que, dans le groupe de contrôle, elle fut de 36,7%. Quatorze semaines après une seconde sommation, les animaux de contrôle infectés auparavant, et ceux infectés récemment, montrèrent des taux de mortalité plus élevés de 53,9 à 68,3%. A l'opposé, dans les groupes prophylactiques, il y eut des taux de mortalité de 17,3 à 25,2%.

Les souris infectées qui reçurent les mêmes doses d'isoniazide pendant quatre semaines et demi avant la sommation, et pendant dix semaines après la sommation, acquérirent quelque degré d'immunité contre une nouvelle sommation. Quatorze semaines après une nouvelle sommation, dans plus de la moitié du groupe prophylactique de souris, les poumons restèrent normaux. Des changements histopathologiques dans tous les organes (poumons, foie, rate, et rein) furent nettement moindres que ceux des animaux de contrôle. A la fin de l'expérience, on n'isola pas de souche de *M. tuberculosis* résistant à l'isoniazide chez aucun des animaux.

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STUDIES OF UNCLASSIFIED MYCOBACTERIA[1]

I. *In Vivo* Response and Morphologic Studies of a Photochromogen

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Introduction

Of the unclassified mycobacteria isolated from patients' specimens, the organisms most capable of causing disease in man and in experimental animals, other than the guinea pig and the chicken, are the photochromogens (1–3). In the reported studies of experimental photochromogenic infection in the mouse (4–6), the inocula that were used were quite large.[2] Progressive infections were observed in the mice inoculated *intravenously* with large concentrations of organisms.

This report is concerned with the *in vivo* response of a photochromogen when approximately *700* viable units were injected *intraperitoneally* into mice, or when approximately *2,000* viable units were inoculated *intraperitoneally* or *subcutaneously* into guinea pigs. The effect of exposure to visible light on the colonial and microscopic morphology of the organisms cultured from the organs of such animals was also observed.

Materials and Methods

The photochromogen was repeatedly isolated from a patient (*WE*) at this hospital who had been diagnosed as having had pulmonary tuberculosis since 1950. The patient had been given various regimens of antituberculous drugs since 1953, including streptomycin and PAS, in the usual dosages for sixteen months: pyrazinamide, isoniazid, and streptomycin for nine months; oxytetracycline and viomycin for eight months; and tetracycline and cycloserine for nine months. An extraperiosteal plombage with Lucite balls was performed in 1954, which were not removed thereafter.

Sputum specimens from this patient were consistently positive for acid-fast bacilli during his hospitalizations, 1953–1955 and from March to November, 1956. All cultures taken from March to October, 1956, were consistently photochromogenic. Although photochromogenicity had been occasionally observed in cultures obtained in 1954 and 1955, the significance of this finding was not recognized at that time. The patient's specimens became negative for acid-fast bacilli after November, 1956. All specimens subsequently taken were also negative until the patient's death in 1959. The culture used throughout this study was obtained in October, 1956. After storage for three months at room temperature in a cabinet, the culture showed cream-white colonies, yellow colonies, and colonies which were half cream-white and half yellow. Exposure to light and incubation at 37°C. for twenty-four hours turned all colonies yellow. Subcultures of this organism have been maintained in the nonchromogenic phase on American Trudeau Society medium or on Löwenstein-Jensen medium, wrapped in aluminum foil, by monthly transfer.[3]

The human strain of *Mycobacterium tuberculosis*, H37Rv, was received through the courtesy of Dr. Steenken from the stock culture collection of mycobacteria maintained at Saranac Lake, New York. This culture has been subcultured monthly on either American Trudeau Society or Löwenstein-Jensen slants since 1954. Every six months, the culture was inoculated into mice intraperitoneally and reisolated from the spleens, eighteen to twenty-one days after inoculation, to check on its mouse pathogenicity.

The 5 per cent hog gastric mucin suspension was prepared as described by Gale and Elberg (7). Groups of 60 Swiss albino mice (Namru strain) were injected intraperitoneally on one side of the abdomen with 0.2 ml. of a 10^{-4} dilution of the photochromogen in its chromogenic phase and with 0.8 ml. of mucin or saline on the other side of the abdomen in the manner described in other publications from this laboratory. Three mice per group were sacrificed daily, and the spleen, omentum, liver, lungs, and kidneys were removed, half for individual culture and half for histopathology. In addition, a group of 20 mice were inoculated intraperitoneally with 0.2 ml. of saline and 0.8 ml. of mucin to serve as controls. Two mice per control group were also sacrificed daily for similar treatment.

Three groups of 8 guinea pigs each[4] were also inoculated. One group received 0.6 ml. of a 10^{-4} dilution of the photochromogen, subcutaneously, into the left thigh: one group was inoculated intraperitoneally with 0.6 ml. of a 10^{-4} photochromogen plus 1.4 ml. of mucin; and the third group was injected intraperitoneally with 0.6 ml. of a 10^{-4}

[1] From the Veterans Administration Hospital, Albuquerque, New Mexico.

[2] Inoculum: 4 mg. dry weight (4); 3 to 30 million viable units (5); 5 to 10 million viable units (6).

[3] A subculture is in the atypical mycobacterial collection maintained by Dr. Ernest Runyon, Veterans Administration Hospital, Salt Lake City, Utah.

[4] Ranging in weight from 750 to 1,100 gm.

TABLE 1

NUMBER OF POSITIVE CULTURES OBTAINED FROM THE ORGANS OF MICE INTRAPERITONEALLY INJECTED WITH 680 VIABLE UNITS OF PHOTOCHROMOGEN AND MUCIN

Day after Inoculation	Liver	Spleen	Omentum	Lung	Kidney	Index of Response*
1	1c† 1c 0	0 0 1c	0 0 0	0 0 0	1c 0 0	2.0
2	2c 2c 1c	2+ 0 2+	0 0 0	0 0 0	0 0 0	4.6
3	4c 1c 1c	4c 2c 1c	1c 2c 0	0 0 0	0 1c 2c	9.7
4	3c 2c ‡	7c 1c 3c	1c 2c 0	0 0 0	1c 0 0	13.8
5	0 ‡ 0	1c ‡ 0	3c 0 0	0 0 0	‡ ‡ 0	14.8
6	1c 2c 1c	3c 7c 1+	1c 3c 0	0 0 0	0 1c 0	19.6
7	0 1c 1+	3c 1c 3c	0 0 0	0 0 0	1c 1c 1c	23.5
8	4c ‡ ‡	2c ‡ ‡	1c ‡ ‡	0 ‡ ‡	0 ‡ ‡	25.0
9	2c 1c 1+	4c 1c 1c	0 1c 0	0 0 ‡	‡ 0 ‡	28.6
10	‡ 1c ‡	0 1c ‡	1c 0 0	0 0 ‡	1+ 2c ‡	30.5
11	‡ ‡ ‡	0 0 0	1c 0 0	0 ‡ ‡	1c ‡ ‡	32.1
12	1c 0 —§	2c 2c —	0 0 —	0 0 —	0 1c —	34.2
13	— — —	— — —	— — —	— — —	— — —	—
14	1c 18c 0 0	1c 1+ 1c 1c	0 2c 0 0	0 0 0 0	0 1c 0 0	38.3
Total	23/39 = 59.0%	27/39 = 69.2%	12/39 = 30.8%	0/39 = 0%	13/39 = 33.3%	38.3

* Index of response = $\frac{\text{cumulative number of positive cultures in all organs}}{\text{total number of organs}} \times 100$

† 1c = 1 colony positive, 0 = negative, 1+, 2+, 3+, 4+, *see* text for explanation.

‡ = contaminated

§ — = not done

photochromogen plus 1.4 ml. of saline. Two guinea pigs per group were sacrificed at ten, twenty, thirty, and forty days, respectively, and the spleen, omentum, liver, lungs, and kidneys were removed for culture.

The organism inocula were prepared by emulsifying the growth of fourteen-day cultures grown on American Trudeau Society medium with steel balls in a balanced salt-casein hydrolysate diluent. Tenfold serial dilutions were made in the same diluent. Viable counts were determined by streaking 0.2-ml. aliquots of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions on each of 4 American Trudeau Society plates, on each of 4 Norite plates, and on each of 4 American Trudeau Society slants, respectively. The inoculum for the mice (0.2 ml. of a 10^{-4} dilution) was 680 viable units; the inoculum for the guinea pigs (0.6 ml. of a 10^{-4} dilution) was 2,040 viable units.

Cultures of the five organs were made individually by mincing finely with scissors and forceps and by inoculating the minced organs on the surface of two tubes of American Trudeau Society medium, respectively.

No attempt at quantitative enumeration of the colonies recovered from each organ was made, although the positive cultures were read according to the following arbitrary standard: 0–25 colonies were counted; 26–50 colonies, 1+; 51–200 colonies, 2+; greater than 200 colonies, but not confluent growth, 3+; confluent growth, 4+.

The cultures were incubated at 37°C. in the dark, wrapped in aluminum foil, and were read weekly. All positives were unwrapped, were exposed to sunlight for one hour at room temperature, and were reincubated for twenty-four hours at 37°C. in the dark. Such positive cultures exposed to light were examined for pigment formation. All cultures were examined for twelve weeks before being discarded as negative.

An "index of response" was derived by dividing the cumulative number of positive cultures obtained from all organs at a time interval after inoculation by the total number of organs cultured × 100. This index reflects the spread of the microorganism from the inoculation site, and is a measure of the *survival* of the organism in the tissues of the host for a given time interval. The index can thus be used as an easy method for comparing the relative survival rates in the animal body of different strains of a microorganism or of different microorganisms which are studied under similar conditions.[5]

RESULTS

Response of Mice to the Photochromogen

Data on the recovery of positive cultures from the organs of mice intraperitoneally inoculated with 680 viable units of photochromogen and *mucin* are presented in table 1. For each organ

[5] Approximately the same number of organisms inoculated, the same number of animals, the same route and mode of inoculation, the same mode of cultivation, and the same method of preparation of the inoculum.

at each day of sacrifice, the results in the left column are those of *mouse 1*, in the middle column, *mouse 2*, and in the right column, *mouse 3*. Animals were not sacrificed on the thirteenth day because of a technical error, but all survivors were sacrificed on the fourteenth day. It may be noted that a positive culture was obtained from at least one organ of one of the three mice at every day of sacrifice. Generally, 1 to 7 colonies were found in the cultures, although occasionally 1+ or 2+ growth was observed within fourteen to eighteen days of incubation. With continued incubation, most of the 1 to 7 colony original isolations had grown to 2+ or 3+ within one week, and the 1+ and 2+ isolations to 3+ and 4+ growth. The total number of positive cultures obtained from each organ was considered an indication of dissemination of the organism: 69 per cent from the spleen, 59 per cent from the liver, 31 per cent from the omentum, and 33 per cent from the kidneys. No positive cultures were obtained from the lungs. The index of response was 38.3.

Contamination occurred in 9 liver cultures, 4 spleen cultures, 2 omentum cultures, 6 lung cultures, and 9 kidney cultures. Positive cultures, if present, would be inhibited or masked by the contaminant. By subtracting these contaminated cultures from the totals, the adjusted total positives per organ were: 77 per cent from the spleen, 77 per cent from the liver, 32 per cent from the omentum, and 43 per cent from the kidneys. The adjusted index of response was 45.5.

Data on the recovery of positive cultures from the organs of mice intraperitoneally injected with 680 viable units of photochromogen and *saline* are presented in table 2. A positive culture from at least one organ of one of the 3 mice was also obtained on each day of sacrifice. Positive cultures of 1 to 6 colonies were found at first observation of these organ cultures. Dissemination of the organisms, as reflected by the total number of positive cultures per organ, was less than that seen when the photochromogen was inoculated with mucin: 42 per cent from the spleen, 18 per cent from the liver, 35 per cent from the omentum, and 10 per cent from the kidneys. As with the mucin-mice, no positive cultures were obtained from the lungs.

TABLE 2

NUMBER OF POSITIVE CULTURES OBTAINED FROM THE ORGANS OF MICE INTRAPERITONEALLY INJECTED WITH 680 VIABLE UNITS OF PHOTOCHROMOGEN AND SALINE

Day after Inoculation	Liver	Spleen	Omentum	Lung	Kidney	Index of Response*
1	0 0 0	0 0 0	0 0 1c†	0 0 0	0 0 0	0.3
2	0 0 0	1c 0 1c	1c 1c 0	‡ 0 0	‡ 0 0	1.7
3	‡ ‡ 1c	1c ‡ 1c	0 0 1c	0 ‡ 0	0 ‡ 0	3.0
4	‡ 3c 8c	1c 0 1c	0 1c 0	‡ 0 0	0 1c 0	5.0
5	‡ 0 0	1c 0 0	1c 0 0	0 0 0	0 0 0	5.7
6	0 5c 0	0 5c 0	0 0 0	0 0 0	0 0 0	6.3
7	‡ 0 0	4c 0 1c	2c 0 0	‡ 0 0	‡ 0 0	7.3
8	3c ‡ 0	2c 3c 2c	1c 3c 3c	‡ ‡ 0	1c ‡ 0	10.0
9	0 0 0	1c 0 0	1c 0 0	0 0 0	0 0 0	10.7
10	0 0 0	1c 0 0	0 10c 0	0 0 0	0 0 1c	11.7
11	0 0 0	0 0 0	0 0 1c	0 0 0	1c 0 0	12.3
12	1c 0 1+	1c 0 1c	0 0 2c	0 0 0	0 0 1c	14.3
13	0 0 1c	0 2c 1c	0 0 0	‡ 0 0	0 0 0	15.3
14	‡ 0 0	‡ 0 0	‡ 0 0	‡ 0 0	‡ 0 0	15.3
15	0 0 0	1c 1c 0	1c 0 0	0 0 0	1c 0 0	16.7
16	0 0 ‡	6c 0 2c	1+ 1c 1c	0 0 0	‡ 0 0	18.3
17	0 3c 0	1c 0 0	0 0 1c	0 0 0	0 0 0	19.3
18	0 0 0	0 0 0	0 1c 0	0 0 0	0 0 0	19.7
19	2c ‡ 0	0 0 0	0 1c 0	0 0 0	0 ‡ 0	20.3
20	1c 0 0	0 0 1c	0 0 0	0 0 0	0 0 0	21.0
Total	11/60 = 18.3%	25/60 = 41.7%	21/60 = 35%	0/60 = 0%	6/60 = 10%	21.0

* *See* legends for table 1.

TABLE 3

POSITIVE CULTURES OBTAINED FROM THE ORGANS OF MICE FOLLOWING INTRAPERITONEAL INOCULATION OF 630 VIABLE UNITS OF H37Rv WITH MUCIN OR SALINE, RESPECTIVELY

Day after Inoculation	Liver	Spleen	Omentum	Lung	Kidney	Index of Response*
	630 H37Rv + Mucin					
3	0 0 0	0 0 0	0 0 0	1e† 0 0	0 —§ —	1.0
6	0 0 0	0 0 0	0 0 0	0 0 0	— — —	1.0
9	0 0 ‡	7e 0 0	0 0 0	0 0 0	0 0 0	2.0
12	‡ 0 0	0 10e 0	0 0 0	0 0 0	0 0 0	3.0
15	0 0 0	0 3+ 0	0 0 0	0 0 0	0 0 0	4.0
18	0 0 0	0 1+ 0	0 1e 0	0 1e 0	0 0 0	7.0
21	0 0 0	2+ 0 9e	0 0 0	0 0 0	0 0 0	9.0
Total	0/21 = 0%	6/21 = 28.4%	1/21 = 4.8%	2/21 = 9.5%	0/16 = 0%	9.0
	630 H37Rv + Saline					
3	‡ 0 0	0 0 0	0 0 0	0 0 0	0 — 0	0
6	0 0 0	0 0 0	0 0 0	0 0 0	0 — 0	0
9	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0
12	0 0 0	0 1e 0	0 0 0	0 0 0	0 0 0	1.0
15	0 0 0	0 0 0	0 0 0	‡ 0 0	0 0 0	1.0
18	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	1.0
21	0 0 0	0 0 0	0 0 0	‡ 0 0	0 0 0	1.0
Total	0/21 = 0%	1/21 = 4.8%	0/21 = 0%	0/21 = 0%	0/19 = 0%	1.0

* *See* legends for table 1.

The index of response was 21.0. Exclusion of the contaminated cultures from the total positive cultures per organ yielded the adjusted figures: 43 per cent from the spleen, 21 per cent from the liver, 36 per cent from the omentum, and 11 per cent from the kidneys. The adjusted index of response was 23.1.

A comparison was made with the results obtained when 630 viable units of H37Rv with mucin or with saline, respectively (table 3), were injected similarly into the same strain of mice.[6] The H37Rv cultures had been grown on American Trudeau Society medium for fourteen days. Emulsions, serial dilutions, and viable counts were made in the same diluent and on the same media as had been done with the photochromogen. The results at twenty-one days after inoculation with H37Rv and mucin were in marked contrast to those obtained with the photochromogen plus mucin fourteen days after inoculation. The total positive cultures per organ of the H37Rv-mucin mice were: 28 per cent from the spleen, 5 per cent from the omentum, 10 per cent from the lungs, and 0 per cent from the liver and kidneys. The index of response was 9.0. Adjustment for contaminated cultures only changed the index of response to 9.2. Similarly, the results observed twenty-one days after inoculation with H37Rv and saline were significantly different from those obtained with the photochromogen and saline at twenty days. The index of response for the H37Rv-saline mice was 1.0 and, after adjustment for contaminated cultures, was still 1.0.

The adjusted index of response thirty-six days after inoculation with 630 H37Rv and mucin was 13.6. At thirty-nine days after inoculation with 630 H37Rv and saline, the adjusted index of response was 10.4. Both indices were much lower than that obtained with 680 viable units of photochromogen and mucin at fourteen days, 45.5; and in the saline series at twenty days, 23.1.

For a further check on the virulence of the culture of H37Rv used by the intraperitoneal route, the number of positive cultures recovered

[6] Data obtained from unpublished work on the *in vivo* effect of intraperitoneal inoculations of both *Candida albicans* and *Mycobacterium tuberculosis*, H37Rv, in mice.

TABLE 4

POSITIVE CULTURES OBTAINED FROM THE ORGANS OF MICE FOLLOWING INTRAPERITONEAL INJECTION WITH 6 × 10^6 H37Rv TOGETHER WITH MUCIN OR SALINE

Day after Inoculation	Liver	Spleen	Omentum	Lung	Kidney	Index of Response*
	6 × 10^6 H37Rv + Mucin					
3	0† 0 0	4+ 0 3+	1+ 0 1+	1c 2+ —‡	— 1+ 0	6.9
6	0 0 ‡	5c 0 3+	0 0 0	0 0 0	0 — 0	8.9
9	1+ 0 0	4+ 0 4+	1c 1+ 3+	1+ ‡ 2+	1c 0 2+	18.7
12	0 0 0	4+ 0 4+	4+ 0 1	0 0 2+	0 0 3+	24.5
15	0 0 0	1+ 4+ 4+	1+ 2+ 3+	3+ 3+ 3+	3+ 1+ 0	35.3
18	0 0 0	3+ 0 0	0 1+ 0	1+ 3+ 0	0 0 7c	40.2
21	0 0 ‡	0 1+ 0	3c 2+ 0	3+ 3+ 0	1c 1+ 0	47.0
Total	1/21 = 4.8%	13/21 = 61.9%	13/21 = 61.9%	12/20 = 60.0%	8/19 = 42.1%	47.0
	6 × 10^6 H37Rv + Saline					
3	0 0 0	1+ 1+ 2+	1c 1+ 3+	5c 0 0	1c 1c —	8.9
6	0 0 1+	0 3+ 3+	0 1+ 2+	0 1c 1c	0 — —	15.7
9	0 3c 0	0 4+ 3+	0 1+ 2+	1c 1+ 0	0 1+ 1+	24.5
12	0 0 0	3+ 0 0	1+ 0 2+	0 0 3c	2+ 0 0	29.4
15	0 1c 0	3+ 4+ 2+	3+ 1+ 0	0 0 0	1+ 1+ 1+	38.3
18	0 0 0	1c 0 0	0 0 0	0 0 0	0 0 0	39.2
21	0 0 0	‡ 3+ ‡	0 0 0	0 0 ‡	‡ 0 0	40.2
Total	3/21 = 14.3%	13/21 = 61.9%	11/21 = 52.4%	6/21 = 28.3%	8/18 = 44.4%	40.2

* *See* legends for table 1.

from the organs of mice injected with 6.3 × 10^6 H37Rv, together with mucin or saline, respectively, was observed (table 4).[6] The index of response (adjusted for contaminated cultures) at twenty-one days of mucin-injected mice was 47.5, and that of the saline series, 41.8. Such indices were very similar to that obtained with the mice injected with photochromogen and mucin.

Many deaths (35 per cent) occurred in the mice injected with photochromogen and mucin as well as in the mucin controls (20 per cent) within forty-eight hours after inoculation. Because of the high death rate in the controls, the deaths of the photochromogen-mucin mice were considered to be nonspecific and possibly due to toxicity of the mucin preparation. This death rate was therefore not used in assessing the pathogenicity of the photochromogen.

Several conclusions were drawn from the total data: (*1*) the photochromogen disseminated more readily and survived better than the human strain, H37Rv, since more positive cultures were recovered from the abdominal organs of the mice injected with this organism than from the organs of mice injected with a comparable concentration of H37Rv. (*2*) The photochromogen was not susceptible to the tuberculostatic material found in mouse livers, which inhibited the growth of H37Rv *in vitro*, as discussed in a previous publication (8). (*3*) The photochromogen could not be cultured from the lungs of injected mice. Few positive cultures were obtained from the lungs of mice injected with a comparable concentration of H37Rv.

Histopathologic Studies

The slides of the organs obtained from the mice injected with the photochromogen plus mucin or saline were reviewed for the presence of granulomas.[7] It was thought that tubercle formation was too much to expect because of the small number of organisms injected; the tissue response to the photochromogen which differs from that of typical tubercle bacilli (3); and the short time interval (twenty days) after inoculation encompassed by this study. Two

[7] By Dr. William Hentel, pathologist at this hospital.

types of tissue response were noted: a granulomatous reaction and a perifocal inflammatory process with massive infiltration of polymorphonuclear leukocytes. The latter reaction was seen early in the course of the development of the infection.

Granulomas were seen in the omentum of all of the mice injected with the photochromogen, regardless of the adjuvant. Granulomas were also seen in the omentum of the mucin controls, from the third to the eighth day after inoculation. No granulomas were seen in the kidney or the spleen sections. The spleen showed congestion and hyperplasia, but no other significant pathology. In a few of the mucin-injected mice, perifocal inflammation was seen in the kidney, but there was no consistent pattern. No unusual pathology was observed in the kidneys of the saline series or in the mucin-control animals. Granulomas were observed in the livers of the mucin-injected mice from the seventh to the fourteenth day, and in the lungs from the ninth to the fourteenth day. Perifocal inflammation was seen in the liver on the first and fifth days, and in the lungs on the first and second days in these mice. In the saline series, granulomas were observed in the liver on the eighth, ninth, eleventh, eighteenth, and twentieth days; and in the lungs on the ninth, eleventh, and twelfth days. Perifocal inflammation was seen in the lungs on the second, third, and fifth days. No pathology was seen in the liver or lungs of the mucin controls except for perifocal inflammation at the fourth and fifth days.

Although the histopathologic results were not too rewarding, the granulomatous response in the omentum, liver, and lungs of the mice which had received the photochromogen plus saline suggest the proliferation of the organism in these tissues. The results obtained in the organs of the mice injected with photochromogen and mucin were considered equivocal because of the granulomas seen in the omentum of the mucin controls. It was also evident that the organisms had disseminated to the lungs, despite the lack of positive cultures from this organ.

Response in Guinea Pigs

No lesions were seen in any of the guinea pigs, regardless of the route of inoculation, except at the site of injection. Positive cultures were obtained from the area of subcutaneous inoculation or from the exterior peritoneal wall twenty days after inoculation and thereafter. One single positive culture was found in the 40 cultured organs of the guinea pigs injected subcutaneously. This was a culture from the spleen, which showed one colony. Three positive cultures were recovered from the 40 organs of the animals injected intraperitoneally with mucin and organisms: one positive liver culture and two positive spleen cultures, each 1 to 5 colonies. Four positive cultures were obtained from the organs of the guinea pigs injected intraperitoneally with saline and organisms: one liver culture, two spleen cultures, and one omentum culture, also 1 to 5 colonies. In this concentration, there was poor dissemination and survival of the photochromogen in the guinea pig.

Morphologic Studies

A summary of the many observations follows:

Yellow pigmentation as a surface phenomenon: Colonies which had become yellow after exposure to visible light and incubation at 37°C. for twenty-four hours were sliced longitudinally to give cross-section slices. The yellow pigment was present only on the convex surface which had been exposed to light, and not in the inner portions of the colonies. Such colony slices, when exposed to light and incubated at 37°C. for twenty-four hours showed yellow pigmentation only on the exposed surfaces. Similarly, when the top of a yellow-pigmented colony was removed, the inner portion of the colony was not pigmented. Such nonpigmented surfaces also became yellow after exposure to light and incubation for twenty-four hours.

Microscopic appearance of the organisms sampled from the yellow-pigmented surface and from the nonpigmented interior of the colony: Microscopic examination of a smear prepared from a colony prior to exposure to light or from the nonpigmented interior of a yellow colony revealed the organisms as uniformly staining, acid-fast, slender rods, 3 to 5 μ long by 0.5 μ wide, with one or two small granules, i.e., typical tubercle bacilli in appearance (figure 1*a*). Examination of a smear, carefully prepared from only the yellow pigmented surface of the same colony, after exposure to light and incubation for twenty-four hours, showed atypical acid-fast organisms: long, broad, acid-fast rods, 9 to 12 μ long by 2 to 3 μ wide, with 5 to 6 large granules which stained more deeply than the

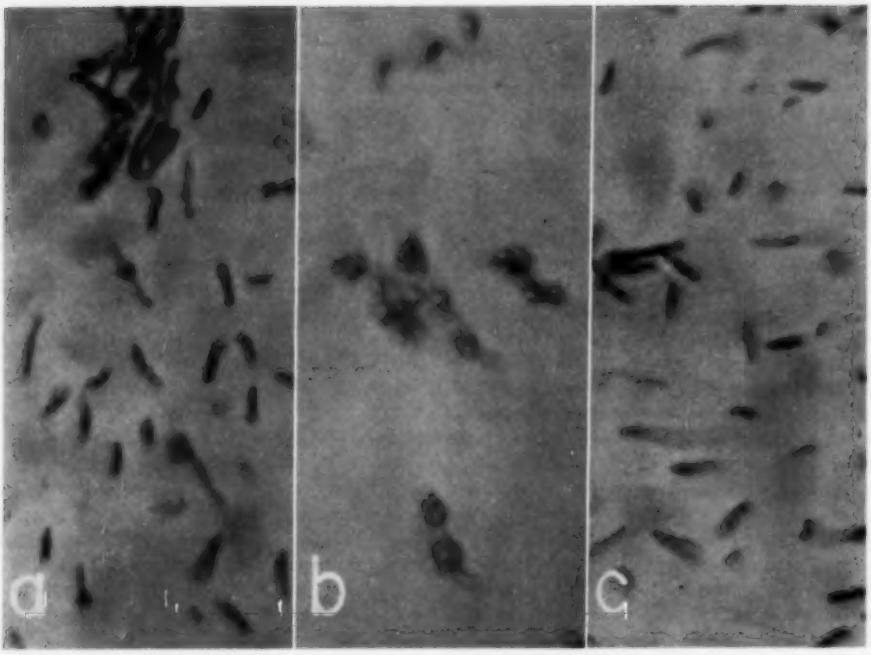

FIG. 1. The microscopic appearance of the photochromogen (× 1400):
a. from the surface of a colony before exposure to light
b. from the surface of the same colony after exposure to light and development of yellow pigmentation
c. from the surface of the same colony after reversion to buff color.

faintly staining rods, and thus presented a beaded or barred appearance (figure 1*b*).

The number of typical and atypical organisms per 10 oil immersion fields of smears prepared from the surface of one colony prior to exposure to light (PCa), and from the yellow surface of the same colony after exposure to light (PCy) was counted:

PCa: 91.1 ± 3.9% typical; 8.9 ± 3.9% atypical

PCy: 7.4 ± 3.3% typical; 92.4 ± 3.3% atypical

Reversion of yellow pigmentation to cream or buff colonies with continued incubation: Colonies which became yellow after exposure to visible light and incubation at 37°C. for twenty-four hours reverted to the nonpigmented form when the culture tubes were wrapped in aluminum foil and were reincubated at 37°C. for ten to fourteen days in the dark. Smears of such reverted colonies showed the typical tubercle bacilli morphology, similar to that seen in smears prepared from the colonies prior to exposure to light (figure 1*c*). Such reverted colonies again showed yellow pigmentation when reexposed to light and incubated at 37°C. for twenty-four hours. Smears from the surface of such colonies showed the atypical acid-fast rods. This reversion, yellow → buff → yellow → buff, et cetera, with the accompanying microscopic changes, was demonstrated ten times with one colony.

Effect of the wavelength of visible light on the pigment formation: A preliminary experiment was done to determine the wavelength of monochromatic light and time of exposure necessary to cause yellow pigmentation.

A Lumetron colorimeter with adjustable filters was used. The colorimeter had been made completely light proof to external light sources. The wavelengths examined were 420λ, 490λ, 530λ, 550λ, 580λ, and 650λ. A series of tubes of American Tru-

TABLE 5

EFFECT OF WAVELENGTH OF VISIBLE LIGHT AND TIME OF EXPOSURE ON THE PRODUCTION OF YELLOW PIGMENTATION

Time of Exposure	Wavelength of Light, in λ					
	420λ	490λ	530λ	530λ	580λ	650λ
minutes						
10	Yellow	Yellow	Yellow	Yellow	Yellow	Cream
20						Cream
30						Yellow
40						Yellow
After 10 days' Incubation at 37°C. in Dark						
10	Yellow	Buff	Buff	Buff	Buff	Cream
20	Yellow					Cream
30	Buff					Buff
40	Buff					Buff
After 20 Days' Incubation at 37°C. in Dark						
10	Yellow	Buff	Buff	Buff	Buff	Cream
20	Buff					Cream
30						Buff
40						Buff

deau Society medium were inoculated with 0.1 ml. of an aliquot of a suspension of the photochromogen. The organism had been grown in Proskauer-Beck medium for fourteen days and frozen in aliquots. The tubes (15- by 125-mm. screw-cap tubes) were selected to fit the cuvette compartment of the colorimeter and to be clean and clear. When sufficient growth had occurred (eighteen days), two tubes per wavelength of light and time interval indicated in table 5 were exposed to the monochromatic light in the colorimeter in a dark room. An area, 2.5 cm. long by 1 cm. wide, of the surface growth was thus illuminated by the light. After exposure to the monochromatic light, the culture tubes were wrapped in aluminum foil in the dark room and incubated at 37°C. for twenty-four hours. The cultures were then examined quickly for yellow pigmentation and rewrapped for continued incubation at 37°C. for ten days in the dark. Examination was quickly made for reversion to the nonpigmented form. The tubes were rewrapped and incubated an additional ten days at 37°C. in the dark.

The results (table 5) indicate that exposure at all wavelengths, 420λ to 580λ, for ten minutes was sufficient to produce yellow pigmentation. However, thirty minutes' exposure at 650λ was required for yellow colonies. Reversion to buff colonies after ten days' incubation occurred with most of the yellow colonies which had been exposed to wavelengths of 490λ to 650λ for ten, twenty, thirty, and forty minutes, and to 420λ for thirty and forty minutes. Twenty days' incubation was necessary for reversion to occur in the yellow colonies which had been exposed to 420λ for twenty minutes, but no reversion was seen in colonies which had been exposed to 420λ for ten minutes.

Miscellaneous observations: Mucoid colonies in large numbers did not produce yellow pigmentation with exposure to light, but retained their original cream color. The reversion process is not an all-or-nothing process. Yellow colonies may show buff or cream papillae, or buff segments, after ten days' incubation in the dark, followed by complete reversion to buff color after continued incubation. Incubation of yellow colonies or buff colonies in the light continuously at room temperature produced orange, orange-red, or red colonies. Similar results have been obtained with 3 other photochromogens isolated at this hospital.

DISCUSSION

The purpose of the *in vivo* studies was to ascertain the pathogenicity for mice of a photochromogen in small concentration, rather than with the large inoculums commonly used. More-

over, the focus of attention was on the response of the mice in the first twenty days rather than at the end of seven to eight weeks which was reported by other investigators (5, 6). Under these circumstances, it could not be expected that the usual manifestations of pathogenicity, such as death of the animal, extensive gross disease and manifest lesions, or other indications of the marked multiplication of the organisms in the tissues, would be observed.

Positive photochromogen cultures were recovered from at least one organ of 33 of the 39 mucin-injected survivors (84.6 per cent) fourteen days after inoculation, and from 39 of the 60 saline-injected mice (65 per cent) twenty days after inoculation, when 680 viable units of photochromogen were injected intraperitoneally into mice. Positive cultures were also obtained from at least one organ of 18 of 21 mucin-injected mice (85.7 per cent) and of 15 of 21 saline-injected mice (71.4 per cent) twenty-one days after inoculation, when 6 million H37Rv were inoculated similarly into the same strain of mice. From these data, it may be inferred that the photochromogen was able to survive as well or better than H37Rv in mouse organs, since approximately 700 photochromogen viable units were as effective in such survival as six million H37Rv viable units.

The question arises as to the pathogenicity of the strain of H37Rv used. At the time when these experiments were done, at least five passages and recovery of the organisms from the spleens of mice had been done, in addition to the monthly subcultures on artificial media. In current work in this laboratory with the same strain of H37Rv, intravenous inoculation of 5×10^5 viable units into another strain of inbred white mice[9] and culture of heart's blood, spleen, liver, omentum, lungs, and kidneys yielded an index of response of 68.3 at thirty days. Intravenous inoculation of 5×10^6 viable units gave an index of response of 28.3 in the same strain of white mice. It is apparent that the H37Rv strain elicited better response in the inbred white mice currently in use than in the inbred Namru mice which were used in the experiments described in this report. Difference in the response of the two mouse strains to inoculations of *Candida albicans* and to both *C. albicans* and *Escherichia coli* was reported by Gale and deVesty (9). Assessment of the response of the inbred mice currently in use to intravenous and intraperitoneal inoculations of photochromogens are in progress and will be reported.

[9] Breeding colony maintained at this hospital and derived from mice originally obtained from Taconic Farms, Germantown, New York.

Summary

A photochromogen repeatedly isolated from a patient with diagnosed pulmonary tuberculosis was tested for its *in vivo* response in mice and in guinea pigs. Seven hundred viable units of the photochromogen disseminated and survived better in the tissues of mice than did 700 viable units of *M. tuberculosis*, H37Rv, and did as well as six million units of H37Rv that were similarly tested. Poor dissemination and survival were observed in the organs of guinea pigs. The photochromogenicity of the organism was only a surface manifestation of the colonies, visible after exposure to visible light. Such pigmented colonies reverted back to nonpigmented colonies after ten to fourteen days' incubation in the dark. Change in the microscopic appearance of the organisms accompanied pigment formation and loss of pigment in the presence and absence of light, respectively. Organisms chosen from nonpigmented and reverted colonies showed microscopic morphology typical of tubercle bacilli, whereas organisms taken from the surface of the pigmented colonies were long, broad, beaded, or barred acid-fast organisms. Red light was less favorable to pigment formation than other wavelengths of visible light.

Sumario

Estudios de las Microbacterias Inclasificadas. I. Respuesta In Vivo y Estudios Morfológicos de un Fotocromógeno

Un fotocromógeno aislado repetidamente de un enfermo en que se había diagnosticado tuberculosis pulmonar fué ensayado en cuanto a su respuesta *in vivo* en ratones y en cobayos. Setecientas unidades viables del fotocromógeno se diseminaron y sobrevivieron mejor en los tejidos de los ratones que 700 unidades viables del *M. tuberculosis* H37Rv, y lo pasaron tan bien como seis millones de unidades del H37Rv que fueron ensayadas en forma semejante. En los órganos de cobayos se observó mala diseminación y sobrevivencia. La fotocromogenicidad del microbio fué puramente una manifestación superficial de las colonias, visible tras la exposición a una luz visible. Esas colonias pigmentadas se volvían de nuevo colonias

no pigmentadas después de diez a catorce días de incubación en las tinieblas. La alteración del aspecto microscópico de los microbios acompañaba a la formación de pigmento y a la pérdida de pigmento en la presencia y la ausencia de luz, respectivamente. Los microbios escogidos de las colonias no pigmentadas y viradas mostraron una morfología microscópica típica de los bacilos tuberculosos, mientras que los tomados de la superficie de las colonias pigmentadas eran gérmenes ácidorresistentes largos y anchos en cuentas o barras. La luz roja fué menos favorable para la pigmentogenia que la luz visible de otros largos de onda.

Resume

Etudes de mycobactéries non classées. I. Réponse in vivo et études morphologiques d'un photochromogène

L'auteur a étudié la réponse *in vivo*, chez des souris et des cobayes d'un photochromogène isolé d'une façon répétée chez un malade diagnostiqué tuberculeux pulmonaire. Sept cent unités viables de photochromogène se disséminèrent et survécurent mieux dans les tissus de souris, que 700 unités viables de *M. tuberculosis*, H37Rv, et aussi bien que six millions d'unités de H37Rv que l'on mit à l'épreuve de la même façon. Une dissémination et une survie médiocres furent observées dans les organes des cobayes. La photochromogénicité de l'organisme fut seulement une manifestation de surface des colonies, visible après exposition à la lumière visible. De telles colonies pigmentées redevinrent non pigmentées après dix à quatorze jours d'incubation dans le noir. Un changement dans l'apparence microscopique des organismes accompagna la formation de pigment et sa perte en présence et en l'absence de la lumière, dans les deux cas respectifs. Des organismes choisis à partir des colonies non pigmentées et des colonies ayant changé, montrèrent une morphologie microscopique typique de bacilles tuberculeux, tandis que des organismes pris à la surface des colonies pigmentées furent longs, larges, en perles, ou des organismes acido-résistants rayés. La lumière rouge fut moins favorable à la formation de pigment, que les autres longueurs d'ondes de lumière visible.

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THE ARYLSULFATASE ACTIVITY OF ACID-FAST BACILLI[1]

I. Investigation of Activity of Stock Cultures of Acid-fast Bacilli

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INTRODUCTION

Although there is a paucity of literature dealing with the arylsulfatase activity of acid-fast bacilli, it is a recognized fact that mycobacteria possess this enzyme. In the opinion of the writers, the main reason for lack of interest in the enzyme is the fact that tripotassium phenolphthalein disulfate, the substrate most commonly used to demonstrate arylsulfatase activity of mycobacteria, is difficult to obtain commercially and generally must be prepared by the individual investigator. A recent communication from Wayne (1), however, reveals that the substrate may be obtained from a source in England.[2]

The enzyme arylsulfatase is capable of splitting the bond between the sulfate group and the aromatic ring in compounds having the general formula $ROSO_3H$, where R represents the aromatic ring structure. Details on the mechanism of action and detection of arylsulfatase activity can be found in publications by Whitehead and associates (2, 3). Those writers showed that saprophytic mycobacteria could be distinguished from tubercle bacilli on the basis of arylsulfatase activity. Wayne and co-workers (4) recently have extended these studies to include many of the aerobic Actinomycetales, while Selkon and Mitchison (5) and Marks and Trollope (6) have used arylsulfatase activity as one of a battery of tests for differential diagnosis of acid-fast bacilli.

The present study was undertaken to compare the degree of arylsulfatase activity of stock cultures of acid-fast bacilli in the presence of various concentrations of substrate.

MATERIALS AND METHODS

The 73 cultures employed in this study had the following distribution: 10 strains of *M. tuberculosis* (including H37Ra), 2 isoniazid-resistant human strains of tubercle bacilli,[3] 4 strains of *M. bovis*, 9 strains of *M. avium*,[4] strain avian Jucho,[5] 2 strains of *M. smegmatis*, 3 of *M. phlei*, one *M. butyricum*, and 8 cultures of *M. fortuitum*.[4] The 34 strains of unclassified acid-fast bacilli included 10 cultures of *M. kansasii*,[6] 4 scotochromogens as well as *M. marianum*,[7] *M. scrofulaceum*,[7] *M. paraffinicum*,[7] *M. sp.* (Locke),[7] and *M. sp.* (ATCC 9626, Bushnell),[7] 8 *Group III* nonphotochromogens,[8] one *Nocardia intracellularis*,[8] and 7 *Group IV* rapid growers.[8] All cultures were grown and maintained on Löwenstein-Jensen egg medium. Prior to use in the test, the cultures were transferred to Tween®-albumin broth (in 15- by 125-mm. screw cap tubes) to obtain uniform well-dispersed suspensions to be used as inocula in the tests; in the case of slowly growing acid-fast bacilli, one-week-old broth cultures were used, whereas two to four days seemed optimal for the rapidly growing mycobacteria.

The substrate, tripotassium phenolphthalein disulfate, was prepared as described by Whitehead and associates (3). A 0.04M aqueous solution of the substrate, sterilized by Seitz filtration, was aseptically added to Tween-albumin broth in the following final concentrations: 0.00025M, 0.0005M, 0.001M, 0.002M, and 0.003M. The action of arylsulfatase on this substrate releases free phenolphthalein which may be detected by the addition of alkaline sodium solutions which form the red-colored disodium salt of phenolphthalein. Although 2N sodium hydroxide was used in the present study to detect free phenolphthalein, it should be noted that an excess of sodium hydroxide may lead to formation of the colorless trisodium salt of phenolphthalein (*see* (7) for discussion on Na salts of phenolphthalein). For this reason, several of the preceding investigators (4, 6) have altered the original procedure of Whitehead and associates and have replaced sodium hydroxide with a less alkaline solution, sodium carbonate; with the latter solution there is less chance for fading of the phenolphthalein color complex.

Replicate tubes of Tween-albumin broth (2 ml.), containing the final concentrations of phe-

[1] From the Tuberculosis Unit, Communicable Disease Center, Atlanta 22, Georgia.

[2] L. Light and Co., Ltd., Colnbrook, Bucks, England.

[3] From the laboratory of Dr. Leon Schmidt, Christ Hospital, Cincinnati, Ohio.

[4] Four of the avian strains (W297–W300) and 8 of the *M. fortuitum* strains from Dr. D. W. Smith, Madison, Wisconsin.

[5] From Dr. K. Takeya, Fukuoka, Japan.

[6] From Dr. E. H. Runyon, Salt Lake City, Utah.

[7] From Shirley McMillen, Chicago, Illinois.

[8] From C. E. Smith, Battey State Hospital, Rome, Georgia.

nolphthalein disulfate previously indicated, were inoculated with one drop (0.05–0.1 ml.) of the appropriate age broth culture of the mycobacteria being tested. Previous studies have shown that these inocula contain 0.04–0.08 mg., wet weight, of organisms (approximately 10^6–10^8 viable units). Inoculated tubes were incubated at 37°C. At weekly intervals for three weeks, cultures were tested for arylsulfatase activity by the dropwise addition of 2N sodium hydroxide. The intensity of the red color of the disodium salt of phenolphthalein was determined visually and recorded as ± to 5+, as noted at the foot of table 1.

Results

For comparative purposes, the enzymatic activity of the various mycobacteria is listed in tables 1, 2, and 3 at the listed substrate concentrations, following incubation periods of one, two, and three weeks, respectively. The most notable observation to be made was the marked lack of arylsulfatase activity in human, bovine, and avian tubercle bacilli at the substrate concentrations and times indicated.

The next and, if substantiated, most salient feature to be noted in the tables is the fact that all of the *Group III* nonphotochromes are arylsulfatase positive in this study, whereas the avian strains are negative. If this differential feature can be repeated and corroborated, this will be one of the few techniques which can be used to distinguish reliably avian tubercle bacilli from the so-called Battey "atypicals" (the unclassified *Group III* nonphotochromogens). This differential test will be the subject of the second paper in this series (8); hence, it will not be discussed further at this time.

All rapidly growing mycobacteria (saprophytes and *M. fortuitum* as well as the unclassified *Group IV*) showed a very strong arylsulfatase activity. It should be noted, however, that at a substrate concentration of 0.00025M, *M. fortuitum* and unclassified *Group IV* myco-

TABLE 1

Effect of Substrate Concentration on Arylsulfatase Activity of Mycobacteria
One Week's Incubation

Species	Number of Cultures	Range of Arylsulfatase Activity* at Indicated Substrate Concentration				
		0.00025M	0.0005M	0.001M	0.002M	0.003M
M. tuberculosis	10	−	−	−	−	−
M. tuberculosis (isoniazid-resistant)	2	−	−	−	−	−
M. bovis	4	−	−	−	−	−
M. avium	9	−	−	−	−	−
Avian Jucho	1	±	1	3	3	2
M. smegmatis	2	− to ±	4	4–5	3–4	3–5
M. phlei	3	−	− to 3	± to 4	1–4	1–3
M. butyricum	1	−	3	4	3	5
M. fortuitum	8	2–3	3–4	4–5	4–5	5
M. kansasii	10	−	− to 1	± to 3	± to 3	1–3
Group II-scotochrome	4	−	−	− to 1	− to 2	− to 2
M. marianum	1	−	−	−	±	−
M. scrofulaceum	1	−	1	1	2	1
M. paraffinicum	1	−	−	±	±	1
M. sp. (Locke)	1	−	−	2	2	3
M. sp. (ATCC 9626)	1	−	−	−	−	−
Group III—Nonphotochrome	8	−	− to 1	− to 1	± to 1	1–2
N. intracellularis	1	−	−	±	1	1
Group IV—rapid grower	7	1–3	3–4	3–4	4–5	4–5

* Degree of arylsulfatase activity as indicated by depth of red following addition of 2N NaOH:
− = No color; no enzymatic activity
± = faint tinge of pink
1 = pale pink
2 = pink
3 = light red
4 = red
5 = deep red: very strong enzymatic activity.

TABLE 2

EFFECT OF SUBSTRATE CONCENTRATION ON ARYLSULFATASE ACTIVITY OF MYCOBACTERIA
Two Weeks' Incubation

Species	Number of Cultures	Range of Arylsulfatase Activity* at Indicated Substrate Concentrations				
		0.00025M	0.0005M	0.001M	0.002M	0.003M
M. tuberculosis	10	–	–	–	–	–
M. tuberculosis (isoniazid-resistant)	2	–	–	–	–	–
M. bovis	4	–	–	–	– to ±	– to 1
M. avium	9	–	–	–	–	–
Avian Jucho	1	2	4	4	4	4
M. smegmatis	2	2–4	4	±–4	4	5
M. phlei	3	±–2	±–3	1–4	2–4	2–5
M. butyricum	1	1	4	4	4	4
M. fortuitum	8	3–4	4–5	5	5	5
M. kansasii	10	– to ±	± to 3	1–3	1–3	1–4
Group II—scotochrome	4	–	± to 2	1–3	1–2	1–3
M. marianum	1	–	–	1	1	1
M. scrofulaceum	1	–	1	1	2	2
M. paraffinicum	1	–	±	1	1	1
M. sp. (Locke)	1	–	2	3	3	4
M. sp. (ATCC 9626)	1	–	–	Contaminated		
Group III—Nonphotochrome	8	– to 1	– to 2	± to 3	1–4	1–4
N. intracellularis	1	–	1	1	2	1
Group IV—rapid grower	7	1–4	1–4	4–5	4–5	5

* *See* Footnote, table 1.

TABLE 3

EFFECT OF SUBSTRATE CONCENTRATION ON ARYLSULFATASE ACTIVITY OF MYCOBACTERIA
Three Weeks' Incubation

Species	Number of Cultures	Range of Arylsulfatase Activity* at Indicated Substrate Concentratons				
		0.00025M	0.0005M	0.001M	0.002M	0.003M
M. tuberculosis	10	–	–	–	– to 1	– to ±
M. tuberculosis (isoniazid-resistant)	2	–	–	–	–	–
M. bovis	4	–	–	– to 3	– to 3	1 to 3
M. avium	9	–	–	–	–	–
Avian Jucho	1	3	4	4	5	5
M. smegmatis	2	4	4–5	5	5	5
M. phlei	3	1–2	1–4	2–4	4–5	3–5
M. butyricum	1	2	4	4	4	5
M. fortuitum	8	3–4	4–5	5	5	5
M. kansasii	10	– to 2	± to 4	± to 4	1–4	1–5
Group II—scotochrome	4	– to 1	± to 3	1–3	2–3	1–3
M. marianum	1	–	±	±	1	1
M. scrofulaceum	1	–	2	1	3	3
M. paraffinicum	1	–	1	2	1	1
M. sp. (Locke)	1	1	3	3	3	No growth
M. sp. (ATCC 9626)	1	(Poor or no growth in entire series)				
Group III—nonphotochrome	8	– to 1	± to 1	± to 4	± to 3	1–4
N. intracellularis	1	1	1	2	3	No growth
Group IV—rapid-grower	7	1–4	3–5	3–5	4–5	5

* *See* Footnote, table 1.

bacteria were consistently positive within the first week of incubation, whereas the known saprophytic mycobacteria were negative. Wayne has stated (1) that it is possible to differentiate readily *M. fortuitum* from such saprophytes as *M. smegmatis* and *M. phlei* on the basis of a two- or three-day arylsulfatase test performed on solid media. These studies are being continued in this laboratory and, if fruitful, should serve as an adjunct to the differential sugar typing of *M. fortuitum* and saprophytic mycobacteria as proposed by Gordon and Smith (9).

Discussion

The present data substantiate the earlier studies of Whitehead and associates (2) and of Wayne and co-workers (4) that mammalian and avian tubercle bacilli may be differentiated from saprophytic mycobacteria on the basis of arylsulfatase activity. It should be noted, however, that prolonged incubation will eventually result in positive enzymatic activity in most mycobacteria. Hence, the apparent lack of arylsulfatase in human, bovine, and avian strains, as demonstrated in this study, is a quantitative and not a qualitative deficiency. This latter fact is further borne out by a careful study of the positive cultures in the three tables. As the substrate concentration increased from 0.00025M to 0.003M and as the bacterial population (and hence the enzyme concentration) increased over the period from one to three weeks, the enzymatic activity increased, as evidenced by a larger amount of free phenophthalein liberated. The liberation of greater amounts of phenolphthalein was indicated by the increase in intensity of the red color produced following the addition of alkaline sodium solutions.

While it is possible to demonstrate arylsulfatase activity in most species of mycobacteria, it should be pointed out that, by using the proper combination of substrate concentration and bacterial inoculum, it is possible to adapt this technique for use in the differential typing of certain groups of mycobacteria. The present investigation suggests that a two-week test at substrate concentrations of either 0.0005M or 0.001M would be helpful in separating mammalian and avian tubercle bacilli from the others in this study. This, then, might be a valuable adjunct to other differential procedures already described (10–12).

A further contribution suggested on the basis of these studies is the differentiation of the avian-Battey complex of mycobacteria. At the present time this is one of the most formidable problems of differential diagnosis for the mycobacteriologist. Culturally, biochemically, and antigenically, avian tubercle bacilli are almost indistinguishable from the Battey type of unclassified acid-fast bacilli. *M. avium* may be distinguished from the Battey strains on the basis of the pathogenicity of the former for chickens, providing small inocula (0.001–0.01 mg.) are used for infection; when inocula in the range of 2–5 mg. are used, however, it is not uncommon for Battey organisms to produce infection in fowl. In this light, the arylsulfatase test may provide a more rapid means for distinguishing these two types of mycobacteria. Studies are continuing on this project and will be reported in the second paper of the series.

Differentiation of *M. fortuitum* from certain saprophytic mycobacteria can be accomplished readily on the basis of their acid production from various sugars. The procedure, however, may take several weeks to perform. The present study confirms the work of Wayne (1) that *M. fortuitum* can be distinguished from such organisms as *M. smegmatis* and *M. phlei* on the basis of a rapid arylsulfatase test. Although not as definitive as the sugar-typing method of Gordon and Smith, the arylsulfatase activity apparently separates *M. fortuitum* and other potentially virulent rapid-growers from known saprophytes and, hence, may be used as a rapid presumptive test for virulent rapidly growing acid-fast bacilli; sugar typing then may be performed for more definite species classification. These studies will be continued and reported in another paper.

It is hoped that the availability of the enzyme substrate from a commercial source will encourage further work on what appears to be a useful addition to techniques currently used for differential typing of mycobacteria.

Summary

The arylsulfatase activity of 73 cultures of acid-fast bacilli was studied under a variety of substrate-time combinations. The data indicate

that a two-week arylsulfatase test, using tripotassium phenolphthalein disulfate concentrations of 0.0005M or 0.001M in Tween-albumin broth, permit differentiation of mammalian and avian tubercle bacilli from all other mycobacteria tested.

There also is a suggestion that arylsulfatase activity may be useful in the differential typing of the avian-Battey group of mycobacteria as well as of certain of the rapidly growing acid-fast bacilli.

Sumario

La Actividad en Arilsulfatasa de los Bacilos Acidorresistentes. I. Investigación de esta Actividad en los Cultivos Stock de Bacilos Acidorresistentes

En varias combinaciones de substrato-tiempo, se estudió la actividad arilsulfatásica de 73 cultivos de bacilos ácidorresistentes. Los datos obtenidos indican que una prueba de dos semanas con arilsulfatasa, usando concentraciones de 0.0005M o 0.001M de bisulfato tripotásico de fenolftaleína en caldo de Tween-albúmina, permite hacer la diferenciación de los bacilos tuberculosos de mamíferos y aves de todas las demás micobacterias comprobadas.

Existe además una indicación de que la actividad arilsulfatásica puede resultar útil en la clasificación diferencial del grupo de microbacterias aviarias-Battey así como de la de los bacilos ácidorresistentes de crecimiento rápido.

Resume

L'activité de l'arylsulfatase des bacilles acido-résistants. I. Investigation de l'activité dans des stocks de cultures de bacilles acido-résistants

Les auteurs étudièrent l'activité de l'arylsulfatase de 73 cultures de bacilles acido-résistants, à l'aide de combinaisons variables du temps et de la substance de base. Le compte-rendu indique qu'un examen de l'arylsulfatase pendant deux semaines, utilisant du disulfate tripotassique de phénolphtaléine à des concentrations de 0,0005 M ou de 0,001 M, dans du bouillon d'albumine de Tween, permit de différencier des bacilles tuberculeux d'oiseaux et de mammifères des autres mycobactéries étudiées.

On suppose aussi que l'activité de l'arylsulfatase peut être utile dans la différenciation du groupe de mycobactéries aviaires Battey, ainsi que certains bacilles acido-résistants poussant rapidement.

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THE ARYLSULFATASE ACTIVITY OF ACID-FAST BACILLI[1]

II. The Differentiation of *Mycobacterium Avium* from the Unclassified Group III Nonphotochromogenic Mycobacteria

GEORGE P. KUBICA AND R. EDWARD BEAM

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INTRODUCTION

In recent years, one of the most difficult problems for mycobacteriologists has been the differential typing of *M. avium* and the *Group III* unclassified acid-fast bacilli. The last-named ones are commonly referred to as Battey strains because of their isolation and description by Crow and associates (1) at Battey State Hospital, Rome, Georgia. In an excellent review of the literature concerning unclassified acid-fast bacilli, Runyon (2) has described the close similarity of Battey strains to *M. avium*.

While temperature of incubation and animal pathogenicity may be used to differentiate these organisms from one another, the investigator must bear in mind that his results may be sharply altered by variations in inoculum size and, in the case of animal pathogenicity studies, in the age of the animals and the route of inoculation. For this reason it is apparent that reliance should not be placed upon one single differential criterion to distinguish *M. avium* from Battey strains.

The preceding paper in this series (3) called attention to the possibility of differentiating *M. avium* and Battey strains on the basis of arylsulfatase activity. The present study involves a more detailed investigation of this previous work, including a study of both stock cultures and a series of unknowns.

MATERIALS AND METHODS

The 46 cultures included in the present study are listed in tables 1 and 2. Of the 10 avian cultures, 6 were stock cultures maintained in this laboratory, the other 4 (W297–W300) were obtained from D. W. Smith, Madison, Wisconsin. Twelve stock strains of unclassified *Group III* nonphotochromogens were employed; 2 of these, "Battey" and *Nocardia intracellularis*, were obtained from C. Edwin Smith, Rome, Georgia; the other 10 were supplied by E. H. Runyon, Salt Lake City, Utah. A packet of 16 unknown strains, comprised of both avian and Battey strains, was received from E. H. Runyon. The classification of these unknown strains, based upon animal pathogenicity and other cultural and biologic characteristics, is recorded in the tables. Another group of 8 Battey strains, prefixed by the letter "T," also were included. All unknown strains were run blind without previous knowledge of their identity.

Cultures were maintained on Löwenstein-Jensen egg medium. Prior to testing, these were transferred to Tween®-albumin broth (15- by 125-mm. screw-cap tubes) to provide well-dispersed suspensions for use as inocula; eight-day-old broth cultures were used in all studies.

The enzyme substrate, tripotassium phenolphthalein disulfate, was prepared as described by Whitehead and associates (4), and final concentrations of 0.001M and 0.002M were added to Tween albumin broth as described in the previous paper (3). Replicate tubes of broth with substrate were inoculated with one drop (0.05–0.1 ml.) of an eight-day-old broth suspension of the organisms being studied. After one and two weeks of incubation, cultures were tested for arylsulfatase activity by the dropwise addition of 1M sodium carbonate. Enzymatic activity was recorded as a variation in intensity of the red color of the disodium salt of phenolphthalein; the color grading is indicated at the foot of table 1.

RESULTS

The data recorded in table 1 indicate the arylsulfatase activity of the stock strains of *M. avium* and unclassified *Group III* nonphotochromogens. The most striking difference to be noted is the lack of arylsulfatase activity in avian strains as contrasted to the variable, but definite, enzymatic activity in Battey strains. This differential enzymatic activity can be demonstrated at either substrate concentration. However, the most clear-cut, distinguishing combination seems to be a two-week reading at 0.002M concentration of phenolphthalein disulfate.

The arylsulfatase activity of 24 unknown strains of acid-fast bacilli are listed in table 2. In column 2 of table 2 the type of each unknown strain is listed, based upon animal pathogenicity and other biologic characteristics. The type

[1] From the Tuberculosis Unit, Communicable Disease Center, Atlanta 22, Georgia.

TABLE 1

ARYLSULFATASE ACTIVITY OF M. AVIUM AND GROUP III NONPHOTOCHROMOGENS

Culture	Type*	Activity at Indicated Substrate Concentration†				Type by Arylsulfatase
		0.001M		0.002M		
		1 Week	2 Weeks	1 Week	2 Weeks	
Avian strains						
Kirchberg	Avian	−	−	−	−	Avian
Sheard	Avian	−	−	−	−	Avian
A-12	Avian	−	−	−	−	Avian
A-64	Avian	−	−	−	−	Avian
A-101	Avian	−	−	−	−	Avian
A-311	Avian	−	−	−	−	Avian
W-297	Avian	−	−	−	−	Avian
W-298	Avian	−	−	−	−	Avian
W-299	Avian	−	−	−	−	Avian
W-300	Avian	−	−	−	−	Avian
Group III Nonphotochromogens						
P-2	Battey	−	±	−	1	Battey
P-7	Battey	−	±	−	±	Battey
P-17	Battey	−	1	±	1	Battey
P-20	Battey	±	1	±	1	Battey
P-23	Battey	3	5	4	5	Battey
P-25	Battey	±	1	1	2	Battey
225-A	Battey	±	1	±	2	Battey
584	Battey	±	2	1	2	Battey
585	Battey	−	±	±	1	Battey
586	Battey	±	2	1	3	Battey
III Battey	Battey	±	±	±	1	Battey
N. intracellularis	Battey	±	1	1	2	Battey

* The type designation in column 2 is based upon growth at various temperatures and animal pathogenicity data

† Degree of arylsulfatase activity as indicated by depth of red following addition of 1M Na_2CO_3 :
− = no color; no enzymatic activity
± = faint tinge of pink
1 = pale pink
2 = pink
3 = light red
4 = red
5 = deep red: very strong enzymatic activity

classification in column 7 is based upon arylsulfatase activity. It will be noted that strains P-50 to P-53 are listed as *M. avium*; on the basis of arylsulfatase activity, P-50, P-52, and P-53 definitely are avian strains, whereas P-51 behaves more like a Battey strain. All of the Battey strains were correctly identified.

DISCUSSION

The present data confirm earlier studies (3) that *M. avium* and unclassified *Group III* nonphotochromogens may be distinguished from one another on the basis of arylsulfatase activity. From this preliminary study, it appears that this is an additional method of distinguishing avian and Battey strains from one another. In the experience of this laboratory, the method certainly is as reliable as animal pathogenicity techniques, provided it is performed as herein described. Then, too, the arylsulfatase technique, which takes only two to three weeks to perform, is much more rapid than animal pathogenicity tests, which may take as long as twelve weeks. Hence, presumptive identification of *M. avium* and Battey strains could be made on the basis of arylsulfatase activity and other rapid tests,

TABLE 2

ARYLSULFATASE ACTIVITY OF 24 UNKNOWN STRAINS OF ACID-FAST BACILLI

Culture	Type*	Activity at Indicated Substrate Concentration†				Type by Arylsulfatase
		0.001 M		0.002M		
		1 Week	2 Weeks	1 Week	2 Weeks	
T-36-0	Battey	±	±	±	±	Battey
T-37-0	Battey	±	1	−	2	Battey
T-38-0	Battey	±	1	±	2	Battey
T-39-0	Battey	±	1	±	1	Battey
T-40-0	Battey	±	1	±	1	Battey
T-41-0	Battey	±	±	±	1	Battey
T-85-0	Battey	±	1	1	1	Battey
T-160-0	Battey	−	±	±	1	Battey
P-39	Battey	±	1	1	2	Battey
P-40	Battey	±	1	±	1	Battey
P-41	Battey	±	1	±	2	Battey
P-42	Battey	±	1	±	1	Battey
P-44	Battey	−	±	±	1	Battey
P-45	Battey	−	±	±	±	Battey
P-46	Battey	−	±	±	1	Battey
P-47	Battey	−	±	−	±	Battey
P-48	Battey	±	1	1	2	Battey
P-49‡	Battey	−	−	−	±	Battey
P-50	Avian	−	−	−	−	Avian
P-51	Avian	±	±	1	1	Battey
P-52	Avian	−	−	−	−	Avian
P-53	Avian	−	−	−	−	Avian
P-54	Battey	−	±	±	2	Battey
P-55	Battey	−	1	±	2	Battey

* Type in column 2 based upon growth at various temperatures and animal pathogenicity.

† *See* footnote to table 1.

‡ Nonchromogenic culture.

whereas the more time-consuming animal pathogenicity tests could be used for final definitive support of the diagnosis. Presumably, in time, the arylsulfatase activity and other cytochemical tests may come to replace animal pathogenicity data as a means of differential typing of certain mycobacteria. Perhaps further studies will show that by increasing substrate concentration it may be possible to distinguish avian and Battey strains in seven to ten days.

It should be emphasized here that, as in all other comparative studies performed in this laboratory, *N. intracellularis* behaves exactly like strains of unclassified *Group III*, and not at all like nocardiae. Investigators at the Communicable Disease Center who have studied this organism along with other nocardiae believe very strongly that *N. intracellularis* definitely is not a Nocardia (5). For these reasons, it is thought that some formal move should be made, as suggested in a recent paper by McMillen (6), to call this organism *Mycobacterium intracellularis* and not *Nocardia intracellularis*.

SUMMARY

The arylsulfatase activity has been studied of 46 cultures of acid-fast bacilli, representing strains of *M. avium* and unclassified *Group III* nonphotochromogens. It has been shown that avian tubercle bacilli may be distinguished readily from Battey strains on the basis of their arylsulfatase activity following two weeks' incubation in the presence of substrate.

SUMARIO

La Actividad en Arilsulfatasa de los Bacilos Acidorresistentes. II. La Diferenciación del Mycobacterium Avium de las Micobacterias No Cromógenas Inclasificadas del Grupo III

Se ha estudiado la actividad arilsulfatásica de 46 cultivos de bacilos ácidorresistentes, que representaban cepas del *M. avium* y no cromógenas del Grupo III inclasificado. Quedó demostrado que pueden distinguirse fácilmente los bacilos tuberculosos aviarios de las cepas de Battey tomando por base su actividad arilusfatásica después de dos semanas de incubación en presencia de subestrato.

RESUME

L'activité de l'arylsulfatase des bacilles acidorésistants. II. La différenciation de Mycobacterium avium du groupe III non-classé de mycobactéries non-photochromogènes.

Les auteurs ont étudié l'activité de l'arylsulfatase dans 46 cultures de bacilles acidorésistants, représentant des souches de *M. avium*, et des souches non-photochromogènes du groupe III non-classé. On a montré que des bacilles tuberculeux aviaires peuvent être distinguées avec fidélité des souches Battey, en se basant sur leur activité d'arylsulfatase, après deux semaines d'incubation en présence d'une substance de base.

Addendum

Since writing this paper, the writers have found that the use of 0.003M substrate provides for a better differentiation of *M. avium* from unclassified *Group III* nonphotochromogens.

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THE ARYLSULFATASE ACTIVITY OF ACID-FAST BACILLI[1]

III. Preliminary Investigation of Rapidly Growing Acid-fast Bacilli

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INTRODUCTION

It is well-known that the enzyme arylsulfatase can be demonstrated in most strains of acid-fast bacilli (1, 2). It has been shown (1–3), however, that, through the use of proper substrate concentration and appropriate choice of incubation time, it is possible to subgroup certain of the mycobacteria on the basis of their arylsulfatase activities. Previous studies have shown that mammalian and avian tubercle bacilli can be distinguished from saprophytic mycobacteria.

It has also been suggested (3, 4) that strains of *M. fortuitum* can be distinguished from saprophytic mycobacteria on the basis of arylsulfatase activity. The present study involves an expansion and more detailed investigation of a portion of a previous report from this laboratory (3) concerning the enzymatic activity of rapidly growing acid-fast bacilli.

MATERIALS AND METHODS

A total of 42 cultures was employed in this study, including 25 stock strains and 17 recently isolated rapidly growing acid-fast bacilli. The stock cultures were distributed as follows: 7 saprophytic mycobacteria, including 3 strains of *M. phlei*, 3 of *M. smegmatis*, and one *M. butyricum*; 9 strains of *M. fortuitum* (8 from D. W. Smith, Madison, Wisconsin, one from E. H. Runyon); 9 strains of unclassified *Group IV* rapid growers (from E. H. Runyon, Salt Lake City, Utah). The 17 recently isolated rapid growers were comprised of saprophytes, *M. fortuitum*, and unclassified *Group IV* rapid growers; the actual identification of these strains, as listed in table 2, was based upon cultural characteristics, animal pathogenicity, and carbohydrate utilization (5). Many of the cultures used in this study had been maintained in frozen aqueous suspensions (6) and were transferred to Löwenstein-Jensen medium just prior to use to ensure purity and viability of organisms. Before use in the actual test, all cultures were transferred to Tween®-albumin broth (15- by 125-mm. screw-cap tubes) to obtain the well-dispersed suspensions used as inocula; the age of broth cultures used in this study was four days.

The enzyme substrate, tripotassium phenolphthalein disulfate, was prepared as described by Whitehead and associates (7), and final concentrations of 0.00025M and 0.001M were added to Tween-albumin broth as described in a previous report (3). Replicate tubes of Tween-albumin broth with substrate were inoculated with one drop (0.05–0.1 ml.) of a four-day-old broth suspension of the cultures being tested. At daily intervals from one to five days post inoculation, cultures were tested for arylsulfatase activity by the dropwise addition of 1M sodium carbonate. The extent of enzymatic activity was recorded as a variation in intensity of the red color of the disodium salt of phenolphthalein; the gradation of color observed is listed at the foot of table 1.

RESULTS

The data in table 1 compare the arylsulfatase activities of stock strains of rapidly growing mycobacteria during the five-day test period. The same comparison for the set of 17 unknowns is provided in table 2. While both substrate concentrations may be utilized to subgroup the rapidly growing acid-fast bacilli, the distinction between strains can best and most rapidly be made with 0.001M substrate in two days.

It will be noted that the saprophytic mycobacteria exhibit little or no enzymatic activity at either substrate concentration, even after five days, whereas strains of *M. fortuitum* are quite strongly positive within two or three days. Another notable, and quite unexpected, observation to be made from this study is the suggested possibility of distinguishing many unclassified *Group IV* rapid growers from *M. fortuitum*. A perusal of these data indicates that the unclassified *Group IV* rapid growers have an arylsulfatase activity intermediate between that of saprophytes and *M. fortuitum*. A glance at table 1 reveals that strain IV-383 behaves more like *M. fortuitum* than like unclassified rapid growers, while IV-156 acts more like a saprophyte. These two cultures will be discussed further in the next section.

As a preliminary practical evaluation of this test, 17 recently isolated wild strains of rapidly growing acid-fast bacilli (designated by the pre-

[1] From the Tuberculosis Unit, Communicable Disease Center, Atlanta 22, Georgia.

TABLE 1

ARYLSULFATASE ACTIVITY OF STOCK STRAINS OF RAPIDLY GROWING ACID-FAST BACILLI

Culture	Enzymatic Activity over 5-day Test Period at Indicated Substrate Concentration*									
	0.00025 M					0.001M				
	1	2	3	4	5	1	2	3	4	5
M. smegmatis	–	–	–	–	–	–	–	±	1	1
M. smegmatis (mouse)	–	–	–	–	–	–	–	–	–	–
M. smegmatis (402)	–	–	–	–	2	–	–	±	2	±
M. phlei	–	–	–	–	–	–	–	–	–	–
M. phlei 8A	–	–	–	–	–	–	–	–	–	–
M. phlei 5	–	–	–	–	–	–	–	–	–	–
M. butyricum	–	–	–	–	–	–	–	–	–	–
IV-156	–	–	–	–	–	–	–	–	–	–
IV-197	–	–	–	–	–	–	–	–	1	1
IV-380	–	–	–	±	1	±	±	1	2	4
IV-383	±	±	1	1	3	1	2	4	5	5
IV-415	–	–	–	±	1	–	±	1	1	3
IV-481	–	±	±	±	1	±	±	2	4	4
IV-518	–	–	±	±	1	±	1	1	3	3
IV-573	–	–	±	±	1	–	±	1	±	3
IV-852	–	–	–	–	1	–	–	±	1	3
F-3	±	±	1	2	4	1	2	4	4	5
F-5	±	±	1	2	2	1	2	4	5	5
F-6	±	1	2	2	4	2	4	5	5	5
F-12	Not done					1	2	4	4	5
F-14	±	1	2	3	2	2	4	5	5	5
F-15	±	1	2	2	3	1	3	5	5	5
F-274	–	±	1	1	2	±	2	4	4	5
F-389	–	1	2	2	2	1	3	5	4	5
F-6841	–	±	1	1	3	1	3	5	5	5

* Degree of arylsulfatase activity as indicated by depth of red following addition of 1M Na_2CO_3 :

– = no color; no enzymatic activity
± = faint tinge of pink
1 = pale pink
2 = pink
3 = light red
4 = red
5 = deep red: very strong enzymatic activity

fix letter "T") were included in the present study. These strains had been identified previously by the carbohydrate utilization scheme of Gordon and Smith (5); results of typing by carbohydrate utilization are listed in column 2 of table 2. Except in a few cases in which the distinction between *Group IV* and *M. fortuitum* was not clear cut, results of the arylsulfatase tests closely paralleled those of the carbohydrate utilization method (*see* last column, table 2). From all indications it appears that the arylsulfatase test may find application as a rapid presumptive test for *M. fortuitum*.

DISCUSSION

The results of this study corroborate data obtained in the first paper of this series (3) and information from Wayne (4).

From all indications it appears that it is possible to use the arylsulfatase test as a presumptive differential technique to distinguish *M. fortuitum* from saprophytic mycobacteria. The differentiation of *M. fortuitum* from unclassified *Group IV* rapid growers, while not as clear-cut as the former, may be possible; perhaps other combinations of substrate concentration-incubation time may be more fruitful.

An interesting observation is the fact that IV-383 behaved exactly like cultures of *M. fortuitum* used in this study. Since IV-383 for a long time was listed as an unclassified rapid grower, the thought immediately occurred that this might in fact be classified as *M. fortuitum*. Carbohydrate utilizations performed on these strains showed that IV-383 reacted exactly like *M. fortuitum*. Further investigation revealed that Edwards and co-workers (8) and Gordon and Mihm (9) also confirmed the classification of this organism as *M. fortuitum*. Communication with Runyon indicates that information on the source and classification of IV-156 is lacking. Hence, the indication, by arylsulfatase activity, that this is a saprophyte may be valid. Further testing is necessary to properly type IV-156.

These data seem to indicate that arylsulfatase activity may prove to be a rapid, reliable technique for distinguishing potential pathogens, such as *M. fortuitum*, from saprophytic mycobacteria. Further practical evaluation of this technique will determine its ultimate worth as a tool for differential typing of rapidly growing acid-fast bacilli.

SUMMARY

The arylsulfatase activity of 42 rapidly growing acid-fast bacilli was checked daily for a period of five days at two different substrate concentrations. It has been shown that strains of *M. fortuitum* may be distinguished from rapidly growing saprophytes and, in some cases, from unclassified *Group IV* rapid growers on the basis of their enzymatic activity following two

TABLE 2

ARYLSULFATASE ACTIVITY OF A SET OF "UNKNOWN" RAPID GROWERS

Culture	Identification*	Enzymatic Activity over 5-Day Test Period at Indicated Substrate Concentration†										Type by Arylsulfatase
		0.00025M					0.001M					
		1	2	3	4	5	1	2	3	4	5	
T-28-0	Fortuitum	±	1	1	2	2	1	2	4	4	4	Fortuitum
T-29-0	Fortuitum	±	±	±	1	−	1	2	4	4	±	Fortuitum
T-34-0	Fortuitum	−	±	±	±	−	±	1	3	3	4	IV-Fortuitum
T-84-0	Fortuitum	−	−	−	−	2	−	1	1	1	2	IV
T-16-9	Saprophyte	−	−	−	−	−	−	−	−	−	−	Saprophyte
T-23-9	Fortuitum	−	1	1	1	3	1	4	4	5	5	Fortuitum
T-25-9	Fortuitum	±	±	1	−	4	1	2	5	5	5	Fortuitum
T-26-9	Fortuitum	±	±	1	±	1	1	2	3	4	4	Fortuitum
T-38-9	IV	−	−	−	±	±	−	±	1	1	2	IV
T-71-9	Fortuitum	Not done					±	1	3	3	4	IV-Fortuitum
T-75-9	IV	−	−	±	1	1	−	1	2	2	4	IV-Fortuitum
T-76-9	IV-Fortuitum	±	1	2	2	3	1	2	4	5	5	Fortuitum
T-129-9	Saprophyte	−	−	−	−	−	−	−	−	−	±	Saprophyte
T-136-9	IV	−	−	±	±	1	±	±	1	2	3	IV
T-142-9	IV	−	±	±	1	3	±	1	2	3	5	IV-Fortuitum
T-148-9	IV	−	±	±	±	1	±	±	±	1	ND‡	IV
T-167-9	IV-Saprophyte	Not done					−	±	±	2	ND	IV-Saprophyte

* Typed by carbohydrate utilization.
† See Footnote, table 1.
‡ Not done.

or three days' incubation in the presence of substrate.

SUMARIO

La Actividad en Arilsulfatasa de los Bacilos Acidorresistentes. III. Investigación Preliminar de los Bacilos Acidorresistentes de Crecimiento Rápido

La actividad arilsulfatásica de 42 bacilos ácidorresistentes de crecimiento rápido fué comprobada diariamente durante un período de cinco días con dos distintas concentraciones de subestratos. Ha quedado demostrado que pueden distinguirse las cepas del *M. fortuitum* de los saprofitos de crecimiento rápido y, en algunos casos, de los crecedores rápidos inclasificados del Grupo IV, tomando por base su capacidad encimática después de dos o tres días de incubación en presencia de subestrato.

RESUME

L'activité de l'arylsulfatase des bacilles acido-résistants. III. Investigation préliminaire sur les bacilles acido-résistants poussant rapidement

Les auteurs ont vérifié quotidiennement l'activité de l'arylsulfatase de 42 bacilles acido-résistants poussant rapidement, pendant une période de cinq jours, à deux concentrations différentes de substance de base. On a montré que les souches de *M. fortuitum* peuvent être distinguées des saprophytes poussant rapidement et, dans certains cas, du groupe IV non-classé de bacilles poussant rapidement, en se basant sur leur activité enzymatique après deux ou trois jours d'incubation en présence d'une substance de base.

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Case Reports

EMPYEMA DUE TO SALMONELLA TYPHIMURIUM[1]

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INTRODUCTION

Among the salmonella, other than typhosa, *Salmonella typhimurium* has been more frequently found in man (1–7). Worth mentioning are the conclusions arrived at by Bennett and Hook (8) and by Hook and associates (9) that some diseases, like malaria, relapsing fever, bartonellosis, viral hepatitis, and sickle-cell anemia, create a special predisposition for the infection with Salmonella.

Sometimes the salmonella show the ability to produce localized complications as well as the ability to invade tissues which previously have been the site of pre-existing disease (8, 10). Pulmonary involvement due to Salmonella is the most frequent complication. Goulder, Kingsland, and Janeway (11) reported that *S. choleraesuis* was responsible for the development of pneumonic symptoms and signs in 30 per cent of the cases. Before 1930, most cases of pulmonary complications were known to be due to *S. paratyphi* A and B, but in 1928 Bullowa (12) was able, for the first time, to detect pulmonary complications due to *S. choleraesuis* in the Harlem hospital.

Although pulmonary involvement due to Salmonella is quite frequent, the invasion of the pleural space by the organism and, as a consequence, the occurrence of purulent pleuritis has been observed rarely up to the present time.

Some cases of empyema due to Salmonella found in the literature seem worth mentioning (10, 13–17). Empyemas due to *S. typhimurium* are a very rare finding. Demanet (16) in 1957 mentioned the report of Clements and colleagues, in 1949, on pericarditis and purulent pleurisy caused by *S. typhimurium* in a child fourteen months old. The same writer in 1957 reported a well-documented case of empyema due to *S. typhimurium*. Seligman and colleagues (5, 6), Saphra (4), and Felsenfeld and Young (2) do not mention a single case of empyema due to *S. typhimurium* in their numerous statistics.

Being unable to find more than a few published cases of empyema due to *S. typhimurium*, the present writers thought it would be of interest to report the following case:

G. T., a 54-year-old Negro male, was found to have a left pleural effusion and a small amount of pulmonary infiltration in the left upper lobe zone in October, 1953. In December, 1954, three sputum smears were positive for acid-fast bacilli. The patient was treated with streptomycin and PAS in two tuberculosis hospitals, and was discharged with medical advice in April, 1956. He was not seen again until August 13, 1958, when he was admitted to Lowman Pavilion of Cleveland City Hospital from the emergency room of that hospital. His chief complaints were diarrhea, nausea, and vomiting of two days' duration. He stated he had vomited six to eight times, and the vomitus consisted of greenish material. He also complained of watery diarrhea fifteen to twenty times a day, without mucus or blood, but associated with some tenesmus and abdominal cramps.

The patient was a vagrant and had been drinking excessively prior to his admission. He was given supportive therapy. He had no gastrointestinal symptoms whatsoever after his admission to the hospital. The patient was given no chemotherapy (none was indicated). He was discharged on September 17, 1958 (with no change in his pulmonary status) for further follow-up observation by serial chest films at the outpatient clinic.

He was admitted to Sunny Acres Hospital on May 8, 1959, because of an increase in the pleural effusion at the left base since his discharge from Lowman Pavilion in September, 1958. On admission, he appeared well-developed, undernourished, and chronically ill.

Examination of the chest revealed flatness and decreased breath sounds in the lower third of the left hemithorax posteriorly. The chest was asymmetrical and the left side was smaller than the right. The liver edge was palpable two finger-breadths below the right costal margin, and was firm, smooth, and nontender. Internal hemorrhoids were present. No sputum specimen could be obtained on admission. Blood morphology and urinalysis were within normal limits. Fasting blood sugar was 96 mg. per 100 ml.; blood urea nitrogen, 7.5 mg. per 100 ml. The albumin-globulin ratio was

[1] From Sunny Acres, Cuyahoga County Tuberculosis Hospital, Cleveland 22, Ohio.

0.76:1, and total serum protein was 7.6 gm. A cephalin flocculation test was negative after twenty-four and forty-eight hours. The patient's vital capacity was 2,000 cc. An admission chest roentgenogram of May 8, 1959, showed haziness at the left base characteristic of pleurisy. The lateral wall of the left hemithorax was drawn inward. In the left apex there were "fibrotic looking shadows" with strands extending down toward the left hilus.

On a left lateral chest roentgenogram of May 11, 1959, haziness was seen at the base, chiefly posteriorly. The oldest available chest film (February 6, 1953) was normal, except for a small calcified patch in the left lower lung field. By October, 1953, extensive pleural density was present at the left base and a small amount of pulmonary infiltration in the left upper lobe. Between October, 1953, and April, 1956, several chest films showed some decrease in the amount of pleural density at the left base and a decrease in the pulmonary infiltration. Serial roentgenograms showed no change from April, 1956, until December 4, 1958, when there was an increase in density in the left base; a further increase in density was noted in this area on the film of April 8, 1959.

A left thoracentesis was attempted on May 22, 1959, without success. A second attempt was made on July 20, 1959. This yielded about 50 ml. of purulent fluid. Culture of this specimen revealed gram-negative bacteria, which were later identified as *Salmonella typhimurium*. The bacteria did not ferment lactose, salicin, or saccharose; they formed acid-gas with dextrose, maltose, and mannitol. They were motile, produced H_2S, were indol negative, urea broth negative, methyl red positive, Voges-Proskauer negative, and utilized citrate. There was positive agglutination with polyvalent Salmonella "O" serum and group "B," also with Salmonella "H" antiserum, group i.

On August 6, 1959, a decortication of the thickened pleura over the lower lobe of the left lung was performed, and the empyema pocket was excised. During the surgery the pleura was found to be thick and calcified. About 20 ml. of purulent material were obtained from the empyema pocket. The empyema fluid was again cultured, and the organisms which were grown again showed the characteristics of *Salmonella typhimurium*. Several urine cultures were sterile. Feces were also examined for *Salmonella typhimurium*, but the result was negative. From both the pleural fluid and scrapings of the pleural pocket, viable *Salmonella typhimurium* was isolated. All three cultures of Salmonella were sent to the Ohio Department of Health Laboratories, Columbus, Ohio, and were identified as *Salmonella typhimurium*. The organism was very susceptible to chloramphenicol. Agglutination tests were made on August 27, 1959. The patient's serum gave the following results: typhoid "H" positive 1/20, typhoid "O" positive 1/160, paratyphoid A (flagellar) positive 1/20, paratyphoid B (flagellar b, 1, 2) positive 1/20.

The patient's postoperative course was uneventful; the left lung expanded well.

Summary

Among the salmonella other than typhosa, *S. typhimurium* is the most frequent cause of infection in man. Empyema thoracis as a complication of this infection is an extremely rare finding. A case of empyema thoracis due to *Salmonella typhimurium* is reported. In this case, the onset of the Salmonella infection could not be determined definitely, and it is not entirely clear whether there had been a tuberculous pleural effusion and that this became infected some four or five years later by Salmonella, or whether the two episodes were entirely separate.

Sumario

Empiema Debido a la Salmonella Typhimurium

Entre las Salmonellas otras que typhosa, la llamada *Salmonella typhimurium* es la causa mas frecuente de infección en el hombre. Empiema como una complicación de esta infección es un hallazgo sumamente raro. En este trabajo se informa sobre un nuevo caso de empiema causado por la *Salmonella typhimurium*.

En el caso aquí descrito el principio de la infección con *Salmonella typhimurium* no pudo ser determinado con certeza, y no es muy claro si ya había existido previamente una efusión pleural tuberculosa y que ésta ha sido infectada con la Salmonella unos 4 ó 5 anos después; o si se trata de dos episodios que han de ser considerados como completamente separados uno del otro.

Resume

Empyème dû à Salmonella typhimurium

Parmi les salmonella et autres typhosa, *S. typhimurium* est la plus fréquente cause de l'infection chez l'homme. L'empyème thoracique, en tant que complication de cette infection, est rencontré très rarement. Les auteurs rapportent un cas d'empyème thoracique dû à *S. typhimurium*. Dans ce cas, le début de l'infection à Salmonella ne put pas être établi d'une façon sure, et on ne sait pas très clairement s'il y eut un épanchement pleural tuberculeux et si celui-ci fut infecté quatre ou cinq ans plus tard par des salmonella, ou si les deux épisodes sont entièrement à part.

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HYPERSENSITIVITY TO ISONIAZID WITH NEUTROPENIA AND THROMBOCYTOPENIA[1]

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Introduction

Isoniazid is the most potent, best tolerated, and safest drug used in the treatment of tuberculosis. When a hypersensitivity reaction occurs during dual- or triple-drug therapy, isoniazid is not usually suspected as the offending agent. The following case with unusual manifestations is therefore reported.

Case Report

A 25-year-old white Army switchboard supervisor was admitted to Fitzsimons General Hospital on March 23, 1960, for evaluation of his asymptomatic pulmonary infiltration. He had had a ten-day acute febrile illness in 1952, shortly after he had started shoveling chicken manure on an Arkansas farm. He was stationed in Korea in 1954, but had no known specific exposure to tuberculosis. There was no history of allergic disease. A chest roentgenogram in 1953 showed many scattered, densely calcified, 3 to 6 mm. pulmonary densities with hilar calcification. Films in February and March, 1960, showed these findings plus a soft, nodular infiltration in the right apex. A first-strength tuberculin test and a 1:1,000 histoplasmin intradermal test were positive. The coccidioidin skin test was negative. Fungal serologic tests, hemogram, and urinalysis were normal. Multiple sputum and gastric examinations for fungi and *Mycobacterium tuberculosis* were negative. A tentative diagnosis of active pulmonary tuberculosis and inactive histoplasmosis was made, and treatment with 500 mg. of isoniazid, 6 gm. of sodium PAS, and 50 mg. of pyridoxine, all twice daily, was begun on the tenth hospital day (figure 1). The isoniazid blood concentration at six hours was 6.4 γ per ml.

On hospital day 49, the patient developed a papular eruption on the palms, followed by malaise and a mild sore throat (figure 2). Fever was noted for the first time on the evening of day 54. Examination the next morning revealed fever, a generalized, nonpruritic maculopapular eruption, exudative tonsillitis on the right, and bilateral posterior and right anterior cervical adenopathy. There were no petechiae, even after a Rumpel-Leed test. The liver and spleen were not palpable or tender. The leukocyte count, which had been normal on hospital day 34, was 900 per cu. mm., with 6 segmented and 3 nonsegmented neutrophils, 89 lymphocytes, and 2 monocytes per 100 cells (figure 3). Platelets were decreased on microscopy; the actual count the following day was 74,000 per cu. mm. Throat culture grew normal flora; blood culture was sterile. The serum bilirubin and heterophil agglutinins were normal. Bone marrow examination revealed normal maturation of the erythroid series, arrest of the myeloid elements at the metamyelocyte stage, and a decrease in the number and fragmentation of megakaryocytes.

Penicillin therapy was instituted. Isoniazid, sodium PAS, and pyridoxine were stopped. Prompt and complete improvement occurred in the patient and in his hemogram. On hospital day 77, pyridoxine was reinstituted; on day 78, isoniazid in low dosage. The patient noted mild nausea and malaise immediately after his first dose of isoniazid. Eosinophilia was noted for the first time that afternoon. On day 82, examination revealed a generalized maculopapular eruption and posterior cervical adenopathy. Isoniazid and pyridoxine were discontinued, with prompt recovery.

To ensure that pyridoxine was not the offender, it was restarted on day 90, followed by sodium PAS on day 93. No untoward reaction was noted to these drugs or later when daily streptomycin was added. The right apical lesion decreased in size. Except as noted above, no other medication was given.

Discussion

The initial reaction in this patient, who was a slow inactivator of isoniazid, was characterized by malaise, rash, adenopathy, fever, and severe neutropenia and thrombocytopenia. No purpura was observed. The tonsillitis may have been due to a secondary infection. Following recovery, reinstitution of isoniazid in lower dosage caused anorexia, malaise, and eosinophilia, followed by adenopathy and a rash. Minimal thrombocytopenia may have recurred. Pyridoxine alone caused no difficulty, nor did sodium PAS.

The ordinary toxic reaction to isoniazid is peripheral neuritis; to streptomycin, eighth-nerve damage; and to PAS and its derivatives, gastrointestinal upset.

Hypersensitive or allergic reactions to all three of these drugs may be similar (1). The reactions usually begin several weeks after the offending drug is first started or promptly after

[1] From Fitzsimons General Hospital, Denver, Colorado.

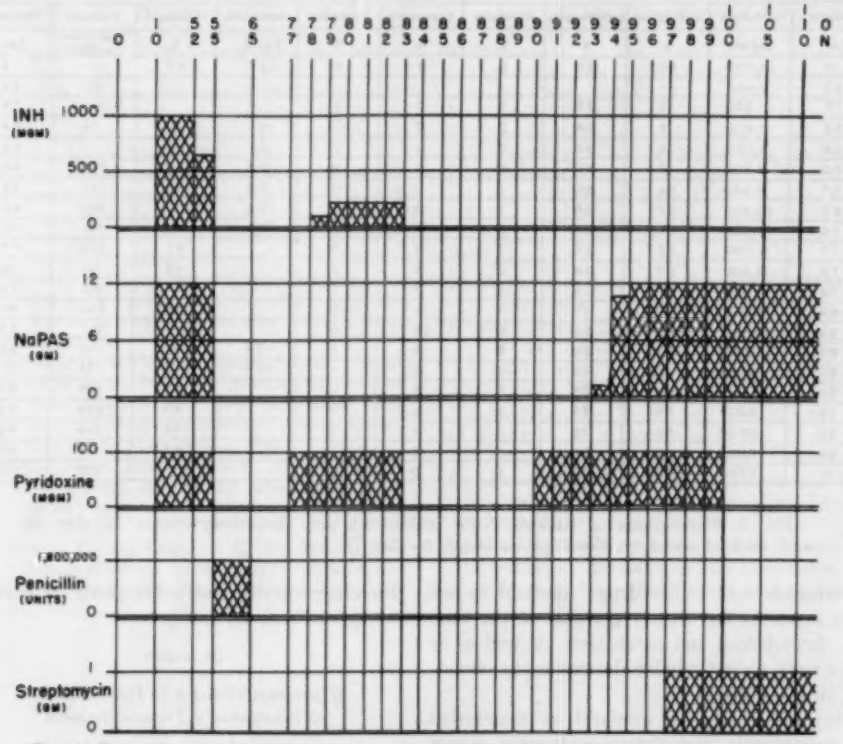

FIG. 1. Drug therapy administered to patient, showing hospital day and drug dosage.

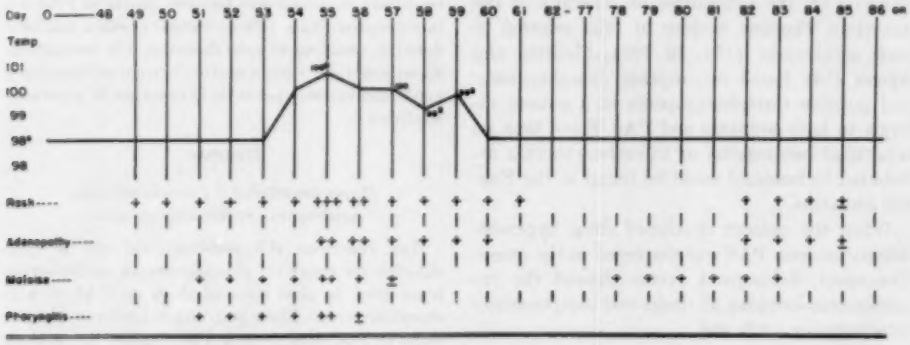

FIG. 2. Temperature curve, significant symptoms, and findings.

its reintroduction. The manifestations of hypersensitivity, in approximate order of descending frequency, are fever, dermatitis, eosinophilia, adenopathy, atypical lymphocytosis, hepatitis, arthralgia, asthma, pulmonary infiltrations, neuritis, convulsions, anemia, leukopenia, and thrombocytopenia (1–14). Fatalities have occurred. Para-aminosalicylic acid and streptomycin each cause hypersensitivity reactions in about 5 per cent of patients being treated for

Hospital	Leukocyte	Neutrophiles	Lymphocytes	Monocytes	Eosinophils	Basophils	Hemoglobin	Hematocrit	Platelets	Hospital
Day	per mm³	%	%	%	%	%	Gm/%	%	per mm³ x 1000	Day
2	10,000	60	33	5	2	0	15.7	47		2
34	7,400								RC	34
55	900	9	89	2	0	0			Reduced	55
56	1,000	6	94	0	0	0	16	48	74	56
58	1,750	8	92	0	0	0	15	46		58
60	2,900	13	80	6	1	0	16.8	49	269	60
62	4,500	44	55	1	0	0	16.8	50	143	62
65	10,400	57	36	7	0	0	15.6		369	65
70	11,000	71	21	6	0	2	15		331	70
77	11,000	62	34	1	3	0		45	210	77
78	12,800	63	28	0	9	0		45	186	78
79	11,800	77	15	0	7	1		44	216	79
80	8,100	77	11	1	11	0		48	232	80
83	10,800	42	29	0	29	0			211	83
85	9,300	53	26	2	18	1	15.4	45	114	85
87	9,000	65	28	0	6	1	15.4	47	185	87
91	11,700	68	24	4	4	0		44	338	91
93	8,800	68	25	1	6	0		46	224	93
95	7,600	56	38	1	4	1		45	210	95
99	8,800	69	29	1	0	1		46	197	99
110	7,700	73	27	0	0	0			202	110

FIG. 3. Hemograms of patient. Note leukopenia and thrombocytopenia on day 55, with gradual recovery. Eosinophilia began on day 78.

tuberculosis with either drug; isoniazid causes such reactions less than 1 per cent of the time (2). Intradermal and patch tests, as well as *in vitro* tests, do not reliably determine the offending drug or drugs.

Hypersensitivity to isoniazid as manifested by neutropenia and thrombocytopenia occurs very rarely, although a report on the toxicity of isoniazid by the Committee on Therapy of the American Thoracic Society in 1953 referred to such occurrences (15). In 1954, Mellette and Agress (14) found neutropenia, lymphocytosis, and possible thrombocytopenia in a patient allergic to both isoniazid and PAS. Since then no reports of neutropenia or thrombocytopenia attributed to isoniazid could be found in the English literature.

When the patient developed drug hypersensitivity, sodium PAS was suspected as the causative agent. Subsequent events showed the importance of stopping all drugs and independently reintroducing each one.

SUMMARY

Hypersensitivity reactions which occur in the course of antituberculous drug therapy are most commonly due to PAS or streptomycin. They may, however, as in this case, be due to isoniazid. The patient in the present report developed thrombocytopenia and neutropenia as part of the hypersensitivity reaction.

SUMARIO

Hipersensibilidad a la Isoniacida con Neutropenia y Trombocitopenia

Las reacciones de hipersensibilidad que aparecen en el transcurso de la farmacoterapia antituberculosa se deben más habitualmente al PAS o a la estreptomicina. Sin embargo, pueden también deberse, como en el caso descrito, a la isoniacida. El enfermo del caso manifestó trombocitopenia y neutropenia como parte de la reacción de hipersensibilidad.

RESUME

Hyper-sensibilité à l'isoniazide avec neutropénie et thrombocytopénie

Les réactions d'hypersensibilité qui se produisent au cours de chimiothérapie antituberculeuse sont le plus souvent dues au PAS et à la streptomycine. Elles peuvent cependant, comme dans ce cas, être dues à l'isoniazide. Le malade dans le cas rapporté, développa une thrombocytopénie et une neutropénie comme manifestation de réaction d'hypersensibilité.

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Notes

THE IDENTIFICATION OF TUBERCLE BACILLI IN 136 SURGICALLY RESECTED LUNG SPECIMENS

The Influence of Preoperative Chemotherapy Utilizing High-Dosage and Low-Dosage Isoniazid

This report is concerned with the ability to recover tubercle bacilli from resected lung specimens of patients who have received drug therapy for active pulmonary tuberculosis. The aim was to determine if there is any difference in the ability to recover tubercle bacilli in lung tissues removed from patients treated with low doses of isoniazid versus patients treated with high doses of the drug.

The use of high doses of isoniazid was begun at this hospital in the fall of 1956. During the two-year period between January, 1957, and January, 1959, 68 patients underwent surgical resections of pulmonary subsegments, segments, or lobes for residuals of tuberculosis. All 68 of these patients were treated daily with 10 to 20 mg. of isoniazid per kg. of body weight. In addition, all patients were given 100 mg. of pyridoxine and 12 gm. of aminosalicylic acid (PAS) daily. Of these 68 patients, 30.8 per cent also received streptomycin.

Prior to the fall of 1956, patients were treated with conventional doses of isoniazid (300 mg. daily) plus 1 gm. of streptomycin every third day. A very small number of patients received PAS instead of streptomycin. Sixty-eight of these patients treated with lower doses of isoniazid and subjected to surgical resection were selected consecutively in a retrospective study.

The following methods were used to identify tubercle bacilli in the removed lung tissues of the 136 patients in both treatment groups: caseous material from the centers of nodules or cavities was smeared directly onto glass slides and examined for the presence of acid-fast-staining bacilli resembling tubercle bacilli. Prepared and stained tissues were mounted on slides, and the presence of bacilli was recorded. In addition, cultures and guinea pigs were inoculated from a suspension of ground tuberculous tissue. A portion of this tissue suspension was digested with trisodium phosphate in an incubator for twenty-four hours and was then inoculated into another culture. The Löwenstein-Jensen medium was used for inoculation of the 136 cases. In the 68 cases of patients treated with high doses of isoniazid, a special oleic-acid-albumin medium was used in addition to the Löwenstein-Jensen medium.

The recovery of tubercle bacilli by all of the techniques outlined above is tabulated in table 1. Tubercle bacilli were seen on immediately prepared slides or recovered in cultures in more than 75 per cent of all removed tissues. The ability to identify organisms was enhanced by approximately 10 to 18 per cent by examination of stained tissues in contrast to direct smears of caseous material. The presence of bacilli on stained smear or tissue section was 4.4 to 11.8 per cent more frequent in the "high-isoniazid" group. Positive cultures were obtained in an approximately equal number of cases in both treatment groups (32.4 to 33.8 per cent). Guinea pigs inoculated with caseous material revealed that the presence of *M. tuberculosis* was 13.2 per cent more frequent in the high-dosage isoniazid treatment group. Only a small number of resected lung tissues in both treatment groups (8 and 9, respectively) did not reveal the presence of any tubercle bacilli. These patients were reported to have cultures positive for *M. tuberculosis* from sputum or gastric contents prior to surgery.

The rates of recovery of *M. tuberculosis* by culture and guinea pig inoculation studies are tabulated in table 2. More than half of all patients had cultures and guinea pig inoculations negative for *M. tuberculosis*. The incidence of both positive culture and guinea pig inoculation from the same tissue was much less than the incidence of both negative culture and guinea pig inoculation, and was approximately 13.2 per cent higher in the high-isoniazid group. A remaining small group of cases was associated with either positive cultures or positive guinea pig inoculations.

In table 3 the incidence of tubercle bacilli on both the direct smear and tissue section was almost the reverse of the situation described in table 2. In table 3, 19.1 to 22.1 per cent of the resected specimens revealed the absence of tubercle bacilli both in tissue sections and on direct smears of caseous material. Approximately half of the specimens were positive by these direct methods of examination. In those cases in which either the tissue or smear of caseous material was positive for *M. tuberculosis*, there was a slightly higher incidence in favor of the group with positive tissue and negative direct smear.

The incidence of the failure to demonstrate viable bacilli in surgically removed lung tissues is summarized in table 4. All tissues contained case-

TABLE 1

RECOVERY OF TUBERCLE BACILLI FROM 136 RESECTED LUNG SPECIMENS

Treatment Group	Tissue Section, Positive for *M. Tuberculosis*	Direct Smear Positive for *M. Tuberculosis*	Culture Positive for *M. Tuberculosis*	Guinea Pig Inoculation Positive for *M. Tuberculosis*	No Bacilli Found
Low-isoniazid (68 cases)	45(66.2%)	38(55.9%)	22(32.4%)	21(30.9%)	8(11.8%)
High-isoniazid (68 cases)	53(78%)	41(60.3%)	23(33.8%)	30(44.1%)	9(13.2%)

TABLE 2

ISOLATION OF TUBERCLE BACILLI IN RESECTED TISSUES BY CULTURE AND GUINEA PIG INOCULATION

Treatment Group	Culture and Guinea Pig Inoculation negative for *M. Tuberculosis*	Culture and Guinea Pig Inoculation Postive for *M. Tuberculosis*	Guinea Pig Inoculation Positive, Culture-Negative for *M. Tuberculosis*	Culture Positive, Guinea Pig Inoculation Negative for *M. Tuberculosis*
Low-isoniazid (68 cases)	39 (57.3%)	13 (19.1%)	8 (11.7%)	8 (11.8%)
High-isoniazid (68 cases)	36 (53%)	22 (32.3%)	9 (13.2%)	1 (1.5%)

TABLE 3

ISOLATION OF TUBERCLE BACILLI IN RESECTED TISSUES BY DIRECT SMEAR AND STAINED TISSUE SECTION

Treatment Group	Tissue Section and Direct Smear Negative for *M. Tuberculosis*	Tissue Section and Direct Smear Positive for *M. Tuberculosis*	Tissue Positive, Smear Negative for *M. Tuberculosis*	Smear Positive, Tissue Negative for *M. Tuberculosis*
Low-isoniazid (68 cases)	15 (22.1%)	30 (44.2%)	15 (22.1%)	8 (11.8)%
High-isoniazid (68 cases)	13 (19.1%)	38 (55.8%)	15 (22.1%)	2 (2.9%)

ous granulomas consistent with a diagnosis of tuberculosis. Viable bacilli were not recovered in 53 to 57.3 per cent of all cases. In the low- and high-isoniazid treatment groups, 8 and 9 tissue specimens, respectively, failed to show the presence of acid-fast staining bacilli in immediately prepared smears or tissue sections. Although cultures and guinea pig inoculations from these aforementioned tissues were also negative, cultures positive for *M. tuberculosis* were obtained from sputum or gastric specimens before surgery.

The incidence of negative cultures and guinea pig inoculations from tissues, the type of chemotherapy, and the duration of preoperative chemotherapy are referred to in table 5. There were fewer culturable bacilli in the tissues of patients receiving low doses of isoniazid when preoperative chemotherapy had been received for less than six months. However, in patients who received treatment for six months or more before surgery, there was little difference in the incidence of viable bacilli in removed tissues. An average of 66 per cent of all 136 tissues examined in the aforementioned two treatment groups failed to demonstrate the presence of viable tubercle bacilli.

* * *

In all phases of the present study there appears to be a slightly higher incidence in the ability to identify tubercle bacilli in the group of patients who were treated with high doses of isoniazid. It

TABLE 4

INCIDENCE OF THE FAILURE TO DEMONSTRATE VIABLE BACILLI

Treatment Group	Culture and Guinea Pig Inoculation Negative for *M. Tuberculosis*	No Bacilli Seen on Growth
Low-isoniazid (68 cases)	39(57.3%)	8
High-isoniazid (68 cases)	36(53%)	9

TABLE 5

RELATIONSHIP BETWEEN THE FAILURE TO RECOVER VIABLE BACILLI AND THE DURATION OF PREOPERATIVE TREATMENT

Number of Months of Treatment	Low-Isoniazid Group		High-Isoniazid Group	
	Number of Cases	Percentage With Culture and Guinea Pig Inoculation Negative for *M. Tuberculosis*	Percentage With Culture and Guinea Pig Inoculation Negative for *M. Tuberculosis*	Number of Cases
1-5	34	47	25	20
6-12	34	67.6	64.5	48

was difficult to evaluate the extent of residual disease in the resected lung tissues of both groups of patients because the study extended over a period of approximately four years. However, it was the writers' general opinion that the extent of disease in those patients treated with high doses of isoniazid was slightly greater than in those patients treated with low doses of isoniazid. The majority of all removed lung specimens contained cavities. With regard to the failure to demonstrate the presence of viable tubercle bacilli, the number of tissue specimens failing to reveal viable bacilli was only 4.3 per cent higher in the low-isoniazid treatment group.

It is worthy of mention that each group of 68 patients was treated with one of two drug regimens. The combination of isoniazid (300 mg. daily) and streptomycin (1 gm. every three days) is considered less effective than the combination of daily isoniazid and PAS.[1]

Some investigators believe that pyridoxine exerts its antineuritis effect simply by lowering the dosage of isoniazid available for antimicrobial effects.[2] This effect may have played a part in influencing the results reported in the high-isoniazid-PAS treatment group. However, the dose of pyridoxine with relation to isoniazid was much lower in the cases reported herein than in the animal experiments of McCune and associates.[2]

The two considerations mentioned in the preceding two paragraphs may have influenced the results of this report. The selection of drug regimens would be in favor of better results in the high isoniazid-PAS group. On the other hand, the antagonism of pyridoxine for biologically active isoniazid may have nullified this advantage. By the methods used herein, it appears that it is not possible to detect any differences between the two drug regimens with relation to the ability to recover viable bacilli from resected lung tissues.

In summary: The use of guinea pigs in addition to cultures increased the incidence of the demonstration of viable bacilli in resected lung specimens by approximately 12 per cent. With regard to the immediate identification of bacilli in removed tissues, the examination of stained tissue sections appeared to be significantly superior to the examination of direct smears of caseous material. There appears to be no significant difference in the ability to recover tubercle bacilli from removed lung tissues of patients treated with high doses of isoniazid, PAS, and pyridoxine versus lower doses of isoniazid plus intermittent streptomycin.

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[1] LIVINGS, D. B.: Trans 15th Conf on Chemother Tuberc, VA, Army & Navy, 1956, p. 20.

[2] MCCUNE, R., *et al.*: Amer Rev Tuberc, 1957, *76*, 1100.

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TOXICITY OF CYCLOSERINE COMBINED WITH ISONIAZID IN THE TREATMENT OF TUBERCULOSIS IN CHILDREN

Fifteen infants and children, whose ages ranged from eight months to thirteen years, were treated with cycloserine[1] combined with isoniazid. The dose of cycloserine was 20 mg. per kg. of body weight and that of isoniazid varied from 5 to 10 mg. per kg. Pyridoxine and other anticonvulsant drugs were not used prophylactically. Weight, appetite, pulse rate, temperature, and respiratory rate were recorded. Blood cell counts and urinalyses were performed initially, at weekly intervals, and later at monthly intervals. Each patient was closely observed by the resident hospital personnel for any change in personality or behavior. The concentrations of cycloserine obtained in the blood of the children were usually more than 20 γ per ml. These concentrations are within the therapeutic range. Very low concentrations may be accounted for by blood samples obtained beyond the peak levels. Although concentrations of cycloserine as high as 35 γ per ml. of serum were obtained, there were no toxic reactions among this closely observed group of children.

The drug concentrations in an initially treated group, between January and June, 1957, are set forth in table 1; and the cycloserine concentrations in the blood of a second group, treated between August, 1957, and May, 1958, are shown in table 2.

In summary: Cycloserine (20 mg. per kg.) and isoniazid (5 to 10 mg. per kg.) were administered together to infants and children with primary tuberculous infections. Concentrations of cycloserine in the blood were determined and found to be within the accepted therapeutic range. Pyridoxine and anticonvulsant drugs were not administered to these patients. There were no toxic effects from

[1] Cycloserine was supplied as Seromycin by Eli Lilly and Company.

TABLE 1

BLOOD CONCENTRATIONS IN THE BLOOD OF CHILDREN TREATED WITH CYCLOSERINE
Initial Group, January to June, 1957

Patient	Age in Years or Months	Weight	½	¾	1/17	1/14	1/21	2/7	2/14	2/21	3/7	3/14	3/21	5/18	5/16	5/12
		kg.														
A.E.	7 years	19.5		10.0	16.0	19.0	26.0	21.0	32.0	22.0	15.0	18.0	17.0	23.5	20.0	13.0
V.H.	7 years	25.5		10.0	8.0*	17.5	15.0	13.0	17.0	17.0	15.0	14.0	17.0	16.0	17.5	8.0*
A.B.	2½ years	15.0	13.0	10.0	19.0	17.5	21.0	21.0	35.0	21.0	28.0	25.0	28.0	23.5	25.5	
M.P.	13 months	9.1		13.0	8.0*	16.5	23.5	14.7	15.0		7.0*					
R.W.	15 months	11.4	20.0	12.0	20.0	23.0	29.0		24.0	28.0		30.0		14.5	25.5	
A.A.	2 years	13.6	10.0	10.0	11.0	17.5	22.5	19.0	34.0	23.0	27.0	27.0	20.5			
J.W.	3 years	13.6				29.5	19.0	19.0	31.0	19.0	31.0	27.0	30.0	18.0	22.5	

* The blood samples were taken at longer intervals than when maximal concentrations occur.

TABLE 2

BLOOD CONCENTRATION IN THE BLOOD OF CHILDREN TREATED WITH CYCLOSERINE
Second Group, August, 1957, to May, 1958

Patient	Age in Years or Months	Weight	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
		kg.									
A.B.	5 years	16.4					17.5	23.0	18.0	17.5	20.0
C.C.	12 months	8.1		33.0	32.0		13.0	21.0	30.0	32.0	19.0
L.H.	3 years	11.4		14.0	42.0		19.0	14.5	15.0	9.0*	12.0
C.L.	8 years	30.9	10.0	6.0*	10.0	10.0		20.5	9.0*	7.0*	7.0*
G.J.	13 months	9.1						24.0	17.0	23.0	23.0
G.W.	13 years	27.3		14.0	24.0		9.3*	20.0	19.0	19.5	
C.R.	8 months	7.8		26.0	64.0		27.2				
E.R.	5 years	15.0		14.0	56.0		27.2				

* The blood samples were taken at longer intervals than when maximal concentrations occur.

these drugs during the two-year period of this study.

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CORTISONE AND TUBERCULIN AS ADJUVANTS TO STREPTOMYCIN AND ISONIAZID IN THE CHEMOTHERAPY OF EXPERIMENTAL TUBERCULOSIS IN THE GUINEA PIG

This report describes experiments done to determine the value of cortisone and tuberculin as adjuvants to the treatment of acute or fulminating tuberculous disease in guinea pigs.

Infection of animals: Albino guinea pigs of 760 gm., average weight, were used. Each animal was given a single subcutaneous injection of a suspension of viable *M. tuberculosis* of murine strain (vole bacilli) and was skin tested with 1:100 Old Tuberculin twenty-one days later; 91 of 96 animals gave positive reactions. Eighty-five of the sensitized animals were weighed and injected intraperitoneally with 0.01 mg. of a virulent strain of *M. tuberculosis* (bovine, Branch) in 0.5 ml. normal saline. They were then divided into three groups of 25 and one group of 10 animals, so arranged that the mean weights of each group were approximately equal. Animals were weighed each week throughout the experiment.

Treatment: The schedule of treatment shown in table 1 was started seven days after intraperitoneal injection of the virulent tubercle bacilli. Streptomycin sulfate, isoniazid and, in *Group B*, cortisone, were injected daily into the muscles of the hind limbs. In *Group C*, doses of purified protein derivative (PPD) were given weekly in increasing dosage, starting with a dose which had been shown in previous experiments to be sufficient to produce twelve to twenty-four hours' anorexia in sensitized guinea pigs (although much larger doses produce no disturbance in nonsensitized animals).

Assessment of results: The effect of treatment was assessed by macroscopic inspection of the peritoneum, pericardium, pleurae, liver, spleen, pancreas, kidneys, lungs, and general lymphatic system. The sites of injection of the murine and the virulent organisms and the regional lymph nodes draining both sites were also inspected.

Infection in each organ of each animal was graded on a 1-to-4 scale by three independent observers; the total number of marks awarded by the three were then added together. If an animal died spontaneously before the end of the experiment, 12 was added, making the total possible score $(3 \times 48) + 12 = 156$.

All of the guinea pigs alive six weeks after treatment had commenced were killed, allocated random numbers, and placed in the postmortem room where they were dissected and then inspected independently by three assessors in the manner described.

Survival: Within four days from the outset of treatment, 2 animals in *Group A* died. Postmortem examination suggested that both had succumbed to an intercurrent pneumonic infection and not to tuberculosis, and they were eliminated from the experiment. A third animal in *Group A* died of tuberculosis at the end of the sixth week of treatment. Nine of the 10 untreated animals (*Group D*) had died spontaneously by the end of the experiment (figure 1).

Weight: All of the animals tended to lose weight initially but, after a full week's treatment, *Groups A, B,* and *C* continued to gain weight (figure 1). As might be expected, *Group D* lost weight steadily.

Infection index: The frequency distribution of the infection index of the animals at postmortem examination is shown in figure 2. The most striking feature of the frequency distribution of the infection index of the animals at postmortem examination is the fact that the guinea pigs of *Group D* are in a totally different range from those in the other three groups. Indeed, so far is their distribution to the right of the other groups that the addition of 12 points for death before the end of the experiment has made no material difference. It can also be seen that, not only is the modal observation for *Group A* higher than for *Groups B* and *C*, but there are 5 more animals with indices higher than 10 in *Group A* than in *Group B*; furthermore, this would still be the case if 12 extra points had not been allocated to the animal in *Group A* that died prematurely. The mean indices for the groups are shown in table 2.

Incidence of abscesses at the site of sensitization: The animals formed two distinct groups with respect to the tissue reaction at the site where the vole bacillus had been introduced subcutaneously. In some animals an abscess had formed; in some no reaction was evident. The incidence of these subcutaneous abscesses is shown in table 3. They were less frequent in the group in which the adjuvant to chemotherapy was tuberculin than in the group in which it was cortisone.

Statistical analysis: A statistical analysis of the findings is presented in table 4, in which it may be seen that, compared with chemotherapy alone, giving cortisone brought about an improvement in the effectiveness of treatment and that this was significant at the 5 per cent level.

* * *

The Seventh Report to the Medical Research Council by the Tuberculosis Chemotherapy Trials Committee[1] showed that daily streptomycin–isoniazid was probably the most effective antibacterial

[1] Seventh Report to the Medical Research Council by the Tuberculosis Chemotherapy Trials Committee, Brit Med J, 1955, *1*, 435.

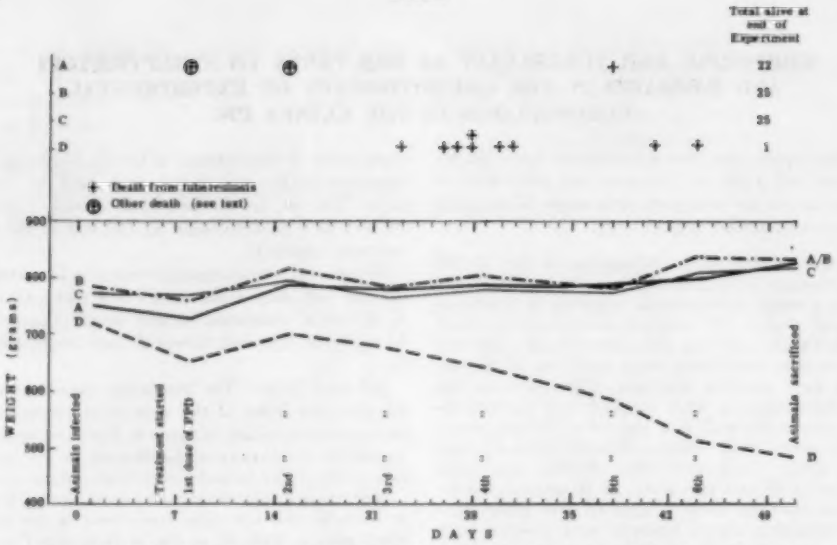

FIG. 1. Details of the mean weights of the four groups, the timing of deaths, and the administration of drugs. Zero on the time scale refers to the day of infection rather than of sensitization, which took place some weeks previously.

TABLE 1

DOSES OF DRUGS RECEIVED BY ANIMALS IN THE FOUR GROUPS

Group	Chemotherapy	Adjuvant
A	Streptomycin sulfate —5 mg./day Isoniazid—2mg./day	None
B	Streptomycin sulfate —5 mg./day Isoniazid—2mg./day	Cortisone—2.5 mg./day
C	Streptomycin sulfate —5 mg./day Isoniazid—2mg./day	Tuberculin* (PPD): First week—0.0075 mg. Second week—0.015 mg. Third week—0.0225 mg. Fourth week—0.03 mg. Fifth week—0.03 mg. Sixth week—0.045 mg.
D	None	None

* Given intraperitoneally forty-eight hours after onset of treatment, and once a week thereafter (*see* figure 1).

treatment then available, and there have been no subsequent reports to the contrary.

It is also widely agreed that steroids facilitate the spread of tuberculosis in man, and the experimental studies of Batten and McCune[3] in the mouse show that, under the influence of corticotropin, cortisone, and hydrocortisone, proliferation of *M. tuberculosis* is increased, the survival time of the animals is shortened, and the lesions in the tissues are modified. The value of such steroids as adjuvants to chemotherapy is still controversial, however.[3] For instance, when Batten and McCune[4] gave mice streptomycin–PAS, isoniazid–pyrazinamide, or isoniazid alone in association with these steroids, they could find no evidence—in terms of survival, pathology, or population counts of *M. tuberculosis* in the various organs—that the antituberculous drug activity was either enhanced or diminished. On the other hand, good results have been claimed for the adjuvant effect of corticotropin and different glucocorticoids in pulmonary tuberculosis[5–7] and in tuberculous meningitis.[8, 9]

[3] BATTEN, J. C., AND MCCUNE, R. M.: Brit J Exp Path, 1957, *38*, 413.

[5] AMERICAN TRUDEAU SOCIETY: Amer Rev Resp Dis, 1960, *82*, 753.

[4] BATTEN, J. C., AND MCCUNE, R. M.: Brit J Exp Path, 1957, *38*, 424.

[8] HOUGHTON, L. E.: Lancet, 1954, *1*, 595.

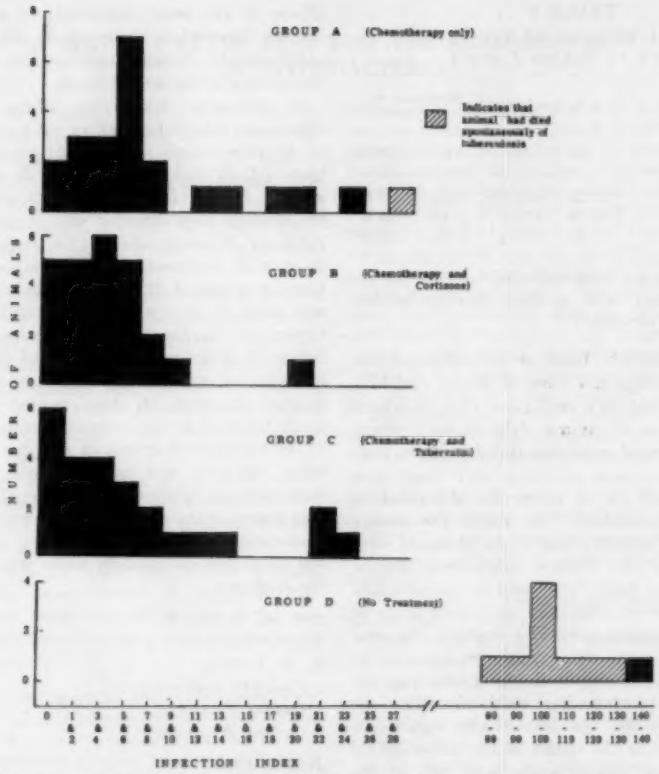
GROUP A (Chemotherapy only)
Indicates that animal had died spontaneously of tuberculosis
GROUP B (Chemotherapy and Cortisone)
GROUP C (Chemotherapy and Tuberculin)
GROUP D (No Treatment)
NUMBER OF ANIMALS
INFECTION INDEX

FIG. 2. Histograms showing the amount of tuberculosis seen macroscopically at necropsy of the guinea pigs in each of the four experimental groups.

TABLE 2

MEAN INDEX OF GENERAL TUBERCULOUS INFECTION IN EACH ANIMAL IN THE FOUR GROUPS

Group	Number of Animals	Infection Index	
		Mean	Standard Error
A	23	7.8	1.62
B	25	3.8	0.84
C	25	6.1	1.47
D	10	102.2	5.73

In pulmonary disease there is some suggestion that these drugs are of greatest value in the acute or fulminating stage of infection, but the evidence is not conclusive. The present writers' results in this study support the value of steroids in acute

TABLE 3

ANIMALS WITH ABSCESSES AT THE SITE OF THE SENSITIZING INOCULATION OF VOLE BACILLUS AT POSTMORTEM EXAMINATION

Group	Total Number of Animals	Animals with Abscesses		
		Number	Per Cent	Standard Error
A	23	9	39	10.1
B	25	12	48	10.0
C	25	6	24	8.5
D	10	8	80	12.6
All animals	83	35	42	5.4

[6] RESEARCH COMMITTEE OF THE TUBERCULOSIS SOCIETY OF SCOTLAND: Brit Med J, 1957, *2*, 1131.

[7] UNITED STATES PUBLIC HEALTH SERVICE: Amer Rev Resp Dis, 1960, *81*, 598.

[8] BULKELEY, W. C. M.: Brit Med J, 1953, *2*, 1127.

[9] ASHBY, M., AND GRANT, H.: Lancet, 1955, *1*, 65.

TABLE 4

STATISTICAL ANALYSIS OF THE RESULTS SHOWN IN TABLES 2 AND 3

Group Comparison	Infection Index		Frequency of Abscesses	
	t	P	*t*	P
A and B	2.25	<0.05	0.63	<0.6
A and C	0.78	<0.5	1.13	<0.3
B and C	1.38	<0.2	1.82	<0.1

Group D has not been included because the effect of treatment is so obvious that no further analysis seems necessary.

infection; indeed, the basis of the difference between these findings and those of Batten and McCune[4] may be that they used a less virulent organism (H37Rv) in an animal (the mouse) which has a higher natural resistance to tuberculosis than the guinea pig.

Recent clinical use of tuberculin is limited to tuberculous meningitis,[10, 11] in which the results compare very favorably with those obtained with other regimens, and chronic tuberculous disease elsewhere in the body.[12, 13] There is no published work on the use of tuberculin to combat acute or subacute tuberculous infections (outside the central nervous system) which might be relevant to the present writers' results in the guinea pig. Although the present results with tuberculin are suggestive, they are not statistically significant. It was intended at the outset of the investigation to follow up the present study with one on the effects of the same combinations of agents on chronic tuberculous infection in the guinea pig; unfortunately, circumstances have so far precluded completion of this second phase.

In summary: Eighty-five albino guinea pigs were made tuberculin positive with a murine strain of *M. tuberculosis* and divided into four groups, three (*A*, *B*, and *C*) containing 25 animals each, and the fourth (*D*) containing 10 animals. All of the animals were infected with a virulent bovine strain of *M. tuberculosis*. One week later, treatment with streptomycin and isoniazid was instituted in groups *A*, *B*, and *C*; in addition, cortisone was given to *Group B* and tuberculin (PPD) to *Group C*. On the grounds of survival, maintenance of body weight, spread of infection, and incidence of subcutaneous abscesses at the site of original infection, all three treated groups fared much better than the untreated animals in *Group D*. Hematogenous spread of the disease was least when cortisone was used, and the incidence of subcutaneous abscesses at the site of sensitization was lowest in the group which received tuberculin. Statistical analysis indicated that the effects of the adjuvants are unlikely to be wholly attributable to chance.

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[10] SMITH, H. V., *et al.*: Tubercle, 1956, *36*, 301.

[11] ACHESON, R. M., AND SMITH, H. V.: Quart J Med, 1958 (n.s. 27), 83.

[12] HOWELLS, C. H. L., AND SWITHINBANK, J.: Tubercle, 1957, *38*, 1.

[13] ACHESON, R. M.: Quart J Med, 1956 (n.s. 25), 159.

[14] The writers are grateful to Dr. R. L. Vollum for his advice and for providing the facilities for the experiment, and to Dr. John V. O. Reid, who helped us in many ways.

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A SIMPLE UREASE TEST FOR THE CLASSIFICATION OF MYCOBACTERIA

Interest in the classification of mycobacteria has been reawakened, with increasing attention to the so-called unclassified mycobacteria. To classify these organisms, pathogenicity for animals, susceptibility to drugs, susceptibility to phages, and specificity of tuberculin reaction have been studied, with productive results in this and other laboratories.[1, 2]

As for the biochemical characteristics, Konno and associates[3, 4] found that human and bovine tubercle bacilli were distinguishable from the other types of acid-fast bacilli by means of the niacin or nicotinamidase test. On the other hand, Toda,[5] Hiroki,[6] and Urabe[7] reported that all strains of avian tubercle bacilli tested were extremely low in urease activity.

The purpose of the present paper is to reconfirm their observations upon the urease activity, using our modified urease test, and to demonstrate that the nonchromogens of unclassified mycobacteria also show very low urease activity. Moreover, from these results, it might be suggested that the urease activity is one of the important characteristics for distinguishing the types of mycobacteria.

Acid-fast bacilli used in this experiment were stock cultures of this laboratory and were: 9 human strains; 4 bovine strains; 12 avian strains, 4 of which were received labeled as avian but have been proved to resemble saprophytic mycobacteria[8-10] (these strains will be described as so-called "avian" bacilli in the following sections); a strain of murine bacilli; 61 strains of unclassified mycobacteria, 7 of which were photochromogens isolated in the United States; 27 strains of nonchromogens, including 4 strains isolated in Japan and 23 strains in the United States; 22 strains of scotochromogens, including 6 strains isolated in Japan and 16 strains in the United States; 43 strains of saprophytic acid-fast bacilli; 12 strains of acid-fast bacilli isolated from dogs, 7 of which were suspected to be human strains and the others, nonchromogens[11]; *M. fortuitum*, *M. scrofulaceum*, *M. marinum*, and *M. paraffinicum*. One strain of *Nocardia asteroides* was also examined. The name of each strain is shown in table 3.

The bacilli were cultivated on Ogawa egg medium until maximal growth. The strains that proved to be negative in the urease test were retested, using young, actively growing bacilli. A modified Van Slyke colorimetric method[12] was used for estimation of urease activity.

Experiment 1. Effect of buffer concentration: An avian Kirchberg strain, two strains of so-called "avian" bacilli (Takeo and Jucho) and *M. phlei* were used. Test solutions were prepared in the following way.

Control buffer 1: 1 M NaH_2PO_4, 10 ml. + 1 M K_2HPO_4, 10 ml. + distilled water, 80 ml., pH 6.7.

Control buffer 2: Tenfold dilution of control buffer 1.

Urea buffer 1: 3 gm. of urea were added to 100 ml. of control buffer 1.

Urea buffer 2: 3 gm. of urea were added to 100 ml. of control buffer 2.

Urea buffer 3: 3 gm. of urea were added to 100 ml. of 100 fold dilution of control buffer 1.

Urea was added after each buffer solution was sterilized by autoclaving.

Three-tenths of a milliliter of bacillary suspension (10 to 20 mg. per ml.) and one drop of 0.1 per cent phenol red solution were added to 3 ml. of each test solution and incubated at 37°C. Observations were made at twenty-four-hour intervals by matching with standard pH solutions.

Experiment 2. Influence of the condition of bacillary suspension: Urea buffer 2 and control buffer 2 were used. A homogeneous suspension of BCG was prepared by grinding with glass beads for five minutes, and a gross suspension was made by simply rubbing the bacilli with a platinum loop on the wall of the test tube. The so-called "avian" Takeo and avian Kirchberg strains were used as positive and negative controls, respectively.

Experiment 3. Urease test of various strains of acid-fast bacilli: One hundred and forty-one strains of acid-fast bacilli and a strain of Nocardia were examined for urease activity, using urea buffer 2. Bacillary suspensions were prepared by the rubbing method, as described above. The color change of the solution was read after three days' incubation at 37°C.

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[11] Toda, T., *et al.*: Amer Rev Resp Dis, 1960, *82*, 414.

[12] Van Slyke, K. K., and Archibald, R. M.: J Biol Chem, 1944, *154*, 623.

The results of *Experiment 1* are shown in table 1. In urea buffer 1, the Kirchberg strain was negative in the urease test, and other strains showed color change in two days of incubation. When urea buffer 2 was used, all strains except the Kirchberg strain showed positive results on the second day of incubation. After a week's incubation, the Kirchberg strain also showed the color change. In using urea buffer 3, the Takeo and Jucho strains showed strong color change within only four hours. All strains but the Kirchberg strain showed strongly positive results within twenty-four hours. From the above mentioned results, three days' incubation (using urea buffer 2) was suitable as a routine test for differentiating acid-fast bacilli. The condition of suspension does not affect the result of this urease test (table 2).

In table 3 the results of *Experiment 3* are shown. The urease test was positive in all human, bovine, and so-called "avian" strains, a murine strain, and all strains of photochromogens. All avian strains and nonchromogens were negative in the tests. As

TABLE 1

EFFECT OF BUFFER CONDITION

Strain	Buffer \ Days after Incubation	1	2	3	4	5	6	7	14
M. phlei	CB	−	−	−	−	−	−	−	−
	UB 1	−	+	+	+++	+++	+++	+++	+++
	UB 2	+	+++	+++	+++	+++	+++	+++	+++
	UB 3	+++	+++	+++	+++	+++	+++	+++	+++
Jucho	CB	−	−	−	−	−	−	−	−
	UB 1	−	+	+	++	+++	+++	+++	+++
	UB 2	++	+++	+++	+++	+++	+++	+++	+++
	UB 3	+++	+++	+++	+++	+++	+++	+++	+++
Takeo	CB	−	−	−	−	−	−	−	−
	UB 1	−	+	+	++	+++	+++	+++	+++
	UB 2	++	+++	+++	+++	+++	+++	+++	+++
	UB 3	+++	+++	+++	+++	+++	+++	+++	+++
Kirchberg	CB	−	−	−	−	−	−	−	−
	UB 1	−	−	−	−	−	−	−	−
	UB 2	−	−	−	−	±	±	+	+
	UB 3	−	+	++	+++	+++	+++	+++	+++

CB: control buffer (M/5 phosphate buffer)
UB 1: M/5 urea*-phosphate buffer
UB 2: M/50 urea*-phosphate buffer
UB 3: M/500 urea*-phosphate buffer

−: pH 6.7
±: pH 6.8 to pH 7.0
+: pH 7.1 to pH 7.3
++: pH 7.4 to pH 7.6
+++: more than pH 7.7

* 3 gm. urea per 100 ml. phosphate buffer.

TABLE 2

INFLUENCE OF CONDITION OF SUSPENSION

Strain	Days after Incubation	1	2	3	4	5	7
BCG	homogeneous suspension	+++	+++	+++	+++	+++	+++
	gross suspension	+++	+++	+++	+++	+++	+++
	Takeo	+++	+++	+++	+++	+++	+++
	Kirchberg	−	−	−	−	−	−

−: pH 6.7
+++: more than pH 7.7

TABLE 3

RESULT OF UREASE TEST OF VARIOUS ACID-FAST BACILLI AND NOCARDIA

Type			Source	Positive		Negative	
Human			Isolated from human	H37Rv, H37Ra, Hori, Frankfurt, Aoyama B, DT, Imamura No. 1, Mizuki, H37Rv, SM100	9		
			Isolated from dogs	Fukuoka 8, Fukuoka 14, Kasuya 13, Kasuya 23, Yame 13, Noogata 70, Mizuma 21-1	7		
Bovine				Ushi 2A, Ushi Miwa, BCG, Venerandi	4		
Murine				Vole	1		
Avian						A71, A62, 4110, Kirchberg, 4121, E38686, Flamingo, Jueki	8
Unclassified Mycobacteria	Photochromogens*			P1, P2, P8, P18, P21, P22, P24	7		
	Nonchromogens	Isolated in Japan	from human			Ueda, Shinkura, Gamou, Koofu	4
			from dogs			Ukiha 1-2, Yakatabaru 3, Mizuma 21-2, Kasuya 24, Jieitai 97	5
		Isolated in the United States*				P3, P7, P17, P20, P23, P25, P39, P40, P41, P42, P43, P44, P45, P46, P47, P48, P49, P50, P51, P52, P53, P54, P55	23
	Scotochromogens	Isolated in Japan			2	Matsumoto, Ishii, Miike, Tomita	4
		Isolated in the United States*			6	P5, P15, P19, P27, P28, P33, P35, P36, P37, P38	10
So-called "avian"				Takeo, Hosoya, Dencho, Jucho.	4		

(*Conclusion of Table on next page*)

TABLE 3—*Continued*

Type	Source	Positive		Negative	
	Saprophyte	BOK, Kaeru Jikei, *M. phlei* (Japanese), *M. phlei* (American), Smegma (Japanese), Smegma (American), Ninpi S, S50B, 607, 106XX, S50R, UrinS, Uehida D14f, 95, ±50, Takeuchi K, S20, M220, CE, y5, R1, OK, T1, Cul, BW, LIA, Furomizu, Trmo, Tie, Grass, Horse, SG1(A), SG2(B), OW1, SG7, SG4, SG1-(A)red, HS3	38	920, Kido yellow, Yos, UW1, OW4	5
	Others	*M. fortuitum*, *M. scrofulaceum*, *M. marinum*	3	*M. paraffinicum*	1
	Nocardia			*N. asteroides*	1

* Stock cultures of unclassified mycobacteria sent from Dr. Runyon

TABLE 4

SUMMARIZED RESULT OF UREASE TEST

Type		Urease Test	Positive Strains/ Tested Strains
Human		+	16/16
Bovine		+	4/4
Murine		+	1/1
Avian		−	0/8
Unclassified mycobacteria	photochromogen	+	11/11
	nonphotochromogen	−	0/32
	scotochromogen	− ~ +	8/22
So-called "avian"		+	4/4
Saprophyte		+ ~ −	38/43
Nocardia		−	0/1

to the scotochromogens, 2 of 6 strains isolated in Japan and 6 of 16 strains in the United States showed positive results. Almost all of the saprophytes, 38 of 43 strains tested, were positive. *M. fortuitum*, *M. scrofulaceum*, and *M. marinum* were positive, whereas *M. paraffinicum* and *Nocardia asteroides* were negative. The results are presented in table 4 in summarized form.

* * *

There have been few methods in routine use for classifying the acid-fast bacilli according to their biochemical properties. Although early studies of Pope and Smith[10] revealed that nicotinic acid is produced in much larger amount by human tubercle bacilli than by bovine bacilli, this property was not applied in classifying the acid-fast bacilli. Recently, Konno devised a method for classification called the "niacin test" by which human tubercle bacilli can be distinguished from the other types of acid-fast bacilli. More recently, bovine tubercle bacilli also were reported to be distinguishable from other acid-fast bacilli, based on their lower activity of nicotinamidase.[4]

There has been controversy concerning the urease activity of various types of acid-fast bacilli.

[10] POPE, H., AND SMITH, D. T.: Amer Rev Tuberc, 1946, *54*, 559.

Corper and Sweny[14] showed that human tubercle bacilli could decompose urea, and avian tubercle bacilli were reported negative in the urease test by Toda,[5] Hiroki,[6] and Urabe.[7] On the contrary, Wohlfeil and associates[15, 16] insisted that *Bacterium pseudotuberculosis rodentium* had urease, but that human tubercle bacilli showed no urease activity. Singer and Cysner[17] reported that only 2 of 65 strains of acid-fast bacilli tested failed to decompose urea.

The results of this experiment seem very clear-cut compared with the results of these earlier reports. The reason may be due to the adequate concentration of the buffer solution used. The change of pH during the test depends, not only on the urease activity, but also on the concentration of buffer solution. When buffer action is too strong, change of pH will rarely be detected; and, when too weak, no difference will be found between active and less active strains.

As shown in table 4, the urease activity of avian tubercle bacilli was found to be very low, and it seems possible to distinguish them from other acid-fast bacilli by the urease test used in this experiment. The observation that all strains of nonchromogens examined in this experiment were negative in the urease test is very interesting. This finding coincides well with the fact that nonchromogens resemble avian tubercle bacilli in many biologic and immunologic properties.[3, 7] All strains of so-called "avian" tubercle bacilli (Takeo, Hosoya, Dencho, and Jucho strains), which were proved to resemble, in every respect, saprophytic mycobacteria examined so far,[8–10] were as strongly positive in the urease test as many of the saprophytic mycobacteria. As is well known of other enzymic activities of bacteria, the urease activity of acid-fast bacilli is known to be different according to the culture age of the bacilli. Therefore, it is necessary to use the young actively growing bacilli.

In summary: A simple urease test for classifying acid-fast bacilli was devised, and 141 strains of Mycobacterium and a strain of Nocardia were examined with this test. The positive strains were all human, bovine, so-called "avian", murine, and photochromogens, some scotochromogens, and most saprophytes. On the contrary, all avian strains, nonchromogens, some scotochromogens, and a few saprophytes were negative in this test.

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[14] CORPER, H. J., AND SWENY, H. C.: J Bact, 1918, *3*, 128.

[15] WOHLFEIL, T., AND WEILAND, P.: Zbl Bakt (Orig), 1937, *138*, 388.

[16] WOHLFEIL, T., AND WOLIENBERG, H.: Zbl Bakt (Orig), 1937, *140*, 281.

[17] SINGER, I., AND CYSNER, E.: Amer Rev Tuberc, 1952, *65*, 779.

THE PRESERVATION OF MYCOBACTERIA BY FREEZING IN VARIOUS DILUENTS

In many routine and research procedures concerned with the bacteriology and immunology of tuberculosis and other mycobacterial diseases, it is necessary to preserve mycobacteria for various reasons, such as subsequent testing of their virulence, susceptibility to antimicrobial agents, or maintaining stock cultures. Continuous subculture of organisms at monthly intervals is time consuming, and progressive loss in viability, virulence, or alterations in their growth behavior may occur.

The preservation of bacteria and the effects of various environmental factors on their viability, virulence, and growth characteristics have been studied by numerous investigators and a number of preservation procedures have been described. Although most of these are satisfactory, many are time consuming, costly, or involve special equipment.

Since a need arose in this laboratory for a better method of preserving mycobacteria than the techniques which were in use, a search of the literature was made to find a procedure which was not only simple, safe, and economical, but one which would also maintain a high degree of viability for several months to several years without significantly affecting the characteristics of these organisms. The freezing procedures described by Heckly,[1] Stern and Tompsett,[2] and more recently by Jones[3] appeared to fulfill these needs. However, since the diluents used in each of these methods were different, it was considered worth while to evaluate this preservation technique using several different diluents.

Diluents: The following five diluents were used for study and were prepared as follows: distilled water; charcoal water[4, 5]: decolorizing carbon,[6] 0.1 gm.; water (distilled), 1,000.0 ml.; aqueous sodium chloride: sodium chloride (USP), 8.5 gm.; water (distilled), 1,000.0 ml.; aqueous albumin[5, 7, 8]: bovine albumin,[9] 1.0 gm.; water (distilled), 1,000.0 ml.; glycerol broth[10]: beef extract,[11] 3.0 gm.; peptone,[11] 10.0 gm.; sodium chloride (USP), 5.0 gm.; glycerol (analytical grade), 50.0 ml.; water (distilled), 950.0 ml.

All of the diluents were adjusted to pH 7.0 and, with the exception of the bovine albumin, were then sterilized for fifteen minutes at fifteen pounds' pressure. The albumin diluent was inactivated by heating in a 56° C. water bath for thirty minutes and was sterilized by Seitz filtration.

Organisms: Two human, 2 bovine, and 2 avian tubercle bacilli, 6 unclassified mycobacteria, and 2 saprophytic acid-fast bacilli were selected for study. The sources of these organisms have been indicated previously.[12, 13] They were transferred to Löwenstein-Jensen and Proskauer-Beck media[14] to maintain stock cultures. In addition, 10 streptomycin-, PAS-, and isoniazid-resistant human tubercle bacilli from the laboratory stock cultures were used to study the effect of freezing on drug resistance. Drug-susceptibility tests were performed by the indirect method described previously.[15]

Before use, smears of each strain were made and stained by the Ziehl-Neelsen acid-fast technique. The organisms also were tested by the thioglycollate,[13] peroxidase,[16] neutral red,[17] serpentine cord,[18] and niacin[19] tests. All of these tests served as controls.

Method of preparing organisms for preservation: Six large colonies of each organism were removed from fourteen-day-old stock cultures of Löwenstein-Jensen medium and placed into each of five separate series of sterile 1-dram, flat-bottom, screw-cap vials containing 2 drops of each diluent. The organisms were then carefully emulsified by means of glass stirring rods and the suspensions were diluted to 2 ml. with their own diluents. A similar series of vials was prepared for

[1] Heckly, R. J.: Amer Rev Tuberc, 1950, *62*, 99.

[2] Stern, K., and Tompsett, R.: Amer Rev Tuberc, 1951, *64*, 696.

[3] Jones, W. D.: Amer J Clin Path, 1957, *27*, 363.

[4] Hirsch, J. G.: Amer Rev Tuberc, 1954, *70*, 989.

[5] Tarshis, M. S., and Elberg, S. S.: Amer Rev Tuberc, 1956, *74*, 84.

[6] Pfanstiel Chemical Co., Waukegan, Illinois.

[7] Davis, B. D., and Dubos, R. J.: J Exp Med, 1947, *86*, 215.

[8] Tarshis, M. S.: Acta Tuberc Scand, 1960, *39*, 76.

[9] Fraction V from bovine plasma, The Armour Laboratories, Kankakee, Illinois.

[10] Corper, H. J., and Cohn, M. L.: Amer Rev Tuberc, 1933, *28*, 856.

[11] Bacto, Difco Laboratories, Inc., Detroit, Michigan.

[12] Tarshis, M. S.: J Lab Clin Med, 1959, *54*, 630.

[13] Tarshis, M. S.: Amer Rev Resp Dis, 1960, *82*, 82.

[14] Tuberculosis Laboratory Methods: VA-Armed Forces Cooperative Study on Chemotherapy of Tuberculosis, Dept. of Medicine and Surgery, Washington, D. C., 1958, pp. 28–29.

[15] Tarshis, M. S.: Amer Rev Tuberc., 1953, *67*, 391.

[16] Tirunarayanan, M. O., and Vischer, W. A.: Amer Rev Tuberc, 1957, *75*, 62.

[17] Dubos, R. J., and Middlebrook, G.: Amer Rev Tuberc, 1948, *58*, 698.

[18] Middlebrook, G., *et al.*: Amer Rev Tuberc, 1947, *56*, 175.

[19] Konno, K., Science, 1956, *124*, 985.

each year of study. All the vials were capped firmly and sealed with pressure-sensitive Scotch masking tape[20] and stored upright in a −20° C. deep freezer.

Methods of determining viability and alteration in strain characteristics: At intervals of one, two, and three years, respectively, the vials were removed from the freezer and allowed to thaw at room temperature. When completely thawed, the suspensions were mixed thoroughly by shaking, and 0.2-ml. aliquots were removed from the vials and inoculated on duplicate sets of Löwenstein-Jensen[14] and Tarshis[21] media. The cultures were then incubated at 37° C. in a nearly horizontal position to permit uniform distribution of the inocula. All tubes were capped loosely for various periods to permit evaporation of excess moisture and were then tightened to prevent drying of the media. Readings were made daily under fluorescent light for four weeks, and the results of viability were recorded in the manner described previously.[21]

To determine what other effects, if any, freezing might have on the organisms, smears of each strain were made and acid-fast stained. The organisms also were tested by the thioglycollate, peroxidase, neutral red, serpentine cord, and niacin tests. The results of these tests were then compared with the results of the same tests performed on each strain before it was preserved by freezing.

To determine whether or not the organisms could also withstand refreezing and rethawing without significantly altering their characteristics, the strains which were frozen for one year were resealed and refrozen. These were rethawed and refrozen after two and three years and then subjected to all of the tests mentioned above. Thus, each strain was frozen and thawed once, and refrozen and rethawed twice, during the three-year test period.

All hazardous procedures were carried out under a bacteriologic safety hood. Each test was repeated once. The final readings represent average results.

Results

The data presented in table 1 summarize the results of the viability determinations on all of the organisms after one, two, and three years of preservation in each diluent at −20° C. It may be seen that the viability of each strain was essentially the same regardless of the diluent used or the period of storage. Although recovery of most of the organisms took somewhat longer after three years of preservation compared with the one- and two-year periods, good growth was still obtained in all instances.

The findings in table 2 summarize the results of the morphology, thioglycollate, peroxidase, neutral red, serpentine cord, and niacin tests on all of the organisms after three years of preservation in each diluent at −20° C. It may be seen that, with several minor exceptions, freezing of the organisms in all five diluents for this period did not alter their reactions to any of these tests. Although some light staining and pleomorphic bacilli were observed in the smears of the Kirchberg avian tubercle bacillus preserved in the sodium chloride, albumin, and glycerol broth diluents, most of the bacilli were identical in morphology to the control organism. Pigmentation of the photochromogenic strain AP-2, preserved in all of the diluents, also was more intense than the control strain. Other than these observations, no significant alterations in any of the other strains were noted.

The positive results of the peroxidase and neutral red tests of the Battey organisms, of the positive serpentine cord test of the saprophyte *M. phlei*, and of the positive niacin test of the Ravenel bovine tubercle bacilli for both the test and control specimens were slight compared with the results of the tests with the human tubercle bacilli, which were moderate to intense. Similar observations have been made previously on unpreserved cultures of these strains.[22]

Similar results were obtained with all of the organisms which were preserved under the same conditions for one and two years and with the strains preserved in all of the diluents for two and three years after they were refrozen and rethawed.

The data in table 3 summarize the results of the streptomycin, PAS, and isoniazid susceptibility tests on the drug-resistant human tubercle bacilli which had been preserved for three years in distilled water at −20° C. It will be noted that the results obtained after freezing these organisms for this period were essentially similar to the results before freezing. Similar drug-susceptibility results also were obtained with all of the organisms which were preserved in the other diluents under the same conditions for one, two, and three years, respectively, and with the strains preserved in all of the diluents for two and three years after they were refrozen and rethawed.

* * *

These observations confirm for the most part those of Heckly,[1] Stern and Tompsett,[2] and Jones,[3] all of whom reported recovery of various strains of mycobacteria without significant alteration after one to forty-two months of preservation in 5 per cent bovine serum albumin,[1] liquid oleic-acid–albumin medium,[2] and tap water[3] at −16° to −34° C. Heckly pointed out that direct freezing of the culture, either in liquid Tween®-albumin or glyc-

[20] Minnesota Mining and Manufacturing Co., St. Paul, Minnesota. No. 202.

[21] Tarshis, M. S.: Amer J Clin Path, 1953, *23*, 661.

[22] Tarshis, M. S.: J Lab Clin Med (in press).

TABLE 1

VIABILITY OF VARIOUS STRAINS OF MYCOBACTERIA AFTER ONE, TWO, AND THREE YEARS OF PRESERVATION IN SEVERAL DIFFERENT DILUENTS AT −20°C.

	Diluents														
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
	First Year					Second Year					Third Year				
Human Tubercle bacilli															
A-1	2++++	2++++	2++++	3++++	2++++	2+++	3++++	4+++	4++++	3+++	3++++	5++++	3+++	2++++	4++++
H37Rv	2++++	2+++	2++++	2++++	2+++	3++++	4+++	3++++	3++++	4++++	3++++	3+++	3++++	3++++	3++++
Bovine tubercle bacilli															
Copenhagen	2+++	2++++	2++++	2++++	2++++	4++++	2+++	5++++	3++++	4++++	5++++	6++++	6+++	6++++	5++++
Ravenel	2++++	2++++	3+++	2++++	2++++	3+++	3++++	4++++	2++++	3++++	5++++	8++++	5++++	6++++	5+++
Avian tubercle bacilli															
Kirchberg	4++++	3++++	2+++	2++++	2++++	3++++	5+++	2++++	4++++	3++++	9++++	6+++	9++++	8++++	9++++
Sheard	2++++	2+++	3++++	2++++	2++++	4++++	2++++	4++++	3+++	4++++	7++++	6++++	8++++	5++++	6+++
Unclassified mycobacteria															
Photochromogens															
AP-1	2+++	2++++	3++++	2++++	2+++	2++++	4++++	5++++	3+++	4++++	6++++	9+++	8++++	7+++	6++++
AP-2	2++++	2++++	2+++	3+++	2++++	3++++	2+++	4+++	3++++	5++++	7+++	6+++	6+++	5++++	9++++
Scotochromogens															
AS-1	2++++	2++++	2++++	2++++	2+++	3++++	3+++	5+++	2++++	2++++	4++++	4++++	7+++	6+++	5+++
AS-2	2+++	2+++	3+++	2++++	2++++	3++++	3++++	4++++	4+++	3+++	5++++	4+++	6++++	4+++	5+++
Battey															
AB-1	2++++	2+++	2++++	2++++	2++++	2++++	2++++	3+++	2++++	3+++	6++++	7+++	6+++	7++++	5++++
AB-2	2++++	2++++	2+++	4++++	2++++	3+++	2+++	3+++	3++++	3++++	5++++	6++++	5+++	6++++	6+++
Saprophytic mycobacteria															
M. smegmatis-a	1++++	1++++	1++++	1++++	1++++	1++++	1++++	2++++	1++++	1++++	1++++	1++++	2++++	1++++	1++++
M. phlei-a	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	1++++	2++++

I = distilled water; II = 0.01% charcoal water; III = 0.85% aqueous sodium chloride; IV = 0.1% aqueous bovine albumin; V = 5% glycerol broth.
Numbers represent the day on which growth first appeared.
+++ to ++++ represent the final degree of growth after four weeks at 37°C.

TABLE 2

RESULTS OF MORPHOLOGY, THIOGLYCOLLATE, PEROXIDASE, NEUTRAL RED, SERPENTINE CORD, AND NIACIN TESTS ON VARIOUS STRAINS OF MYCOBACTERIA AFTER THREE YEARS OF PRESERVATION IN SEVERAL DIFFERENT DILUENTS AT −20° C.

Strain	Morphology Test						Thioglycollate Test						Peroxidase Test						Neutral Red Test						Serpentine Cord Test						Niacin Test					
	Diluents																																			
	1	2	3	4	5	C	1	2	3	4	5	C	1	2	3	4	5	C	1	2	3	4	5	C	1	2	3	4	5	C	1	2	3	4	5	C
Human tubercle bacilli																																				
A-1	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H37Rv	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bovine tubercle bacilli																																				
Copenhagen	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Ravenel	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*
Avian tubercle bacilli																																				
Kirchberg	+	+	+	+	+	+†	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sheard	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Unclassified mycobacteria																																				
Photochromogens																																				
AP-1	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AP-2	+	+	+	+	+	+‡	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Scotochromogens																																				
AS-1	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AS-2	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Battey																																				
AB-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*	+	+	+	+	+	+*	−	−	−	−	−	−	−	−	−	−	−	−
AB-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*	+	+	+	+	+	+*	−	−	−	−	−	−	−	−	−	−	−	−
Saprophytic mycobacteria																																				
M. smegmatis-a	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
M. phlei-a	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+*	−	−	−	−	−	−

1 = distilled water; 2 = 0.01% charcoal water; 3 = 0.85% aqueous sodium chloride; 4 = 0.1% aqueous bovine albumin; 5 = 3% glycerol broth; C = control.

+ = positive (also indicates that the results of the test strains were essentially similar to the results of the control organisms); − = negative.

* The reactions of these control organisms, and the test strains preserved in all of the diluents, were slight compared with the results of the human tubercle bacilli which were moderate to intense.

† Some light staining and pleomorphic bacilli were observed in the smears of this strain preserved in diluents 3, 4, and 5.

‡ Pigmentation of this strain, preserved in all of the diluents, was more intense than the control.

TABLE 3

RESULTS OF STREPTOMYCIN, PARA-AMINOSALICYLIC ACID, AND ISONIAZID-SUSCEPTIBILITY TESTS ON DRUG-RESISTANT HUMAN TUBERCLE BACILLI BEFORE AND AFTER THREE YEARS OF PRESERVATION IN DISTILLED WATER AT −20° C.

Strain of *M. Tuberculosis*	Before			After			Before			After			Before			After		
	Streptomycin (γ per ml.)						PAS (γ per ml.)						Isoniazid (γ per ml.)					
	None	10	100	None	10	100	None	10	100	None	10	100	None	5	10	None	5	10
M-1	4+	4+	2+	4+	4+	3+	4+	4+	3+	4+	4+	2+	4+	4+	3+	4+	4+	2+
M-2	4+	4+	3+	4+	4+	3+	4+	4+	3+	4+	4+	3+	4+	4+	2+	4+	4+	1+
M-3	4+	4+	3+	4+	4+	2+	4+	3+	2+	4+	4+	2+	4+	3+	2+	4+	3+	2+
M-4	4+	4+	2+	4+	4+	2+	4+	4+	2+	4+	4+	2+	4+	3+	1+	4+	3+	1+
M-5	4+	3+	2+	4+	3+	2+	4+	4+	4+	4+	4+	4+	4+	4+	3+	4+	4+	3+
M-6	4+	4+	4+	4+	4+	4+	4+	3+	1+	4+	3+	2+	4+	3+	1+	4+	3+	1+
M-7	4+	3+	3+	4+	4+	3+	4+	2+	2+	4+	2+	2+	4+	4+	4+	4+	4+	4+
M-8	4+	2+	1+	4+	2+	1+	3+	2+	1+	4+	2+	1+	3+	1+	1+	3+	1+	1+
M-9	4+	3+	1+	4+	2+	1+	4+	4+	4+	4+	4+	4+	4+	3+	3+	4+	3+	3+
M-10	3+	2+	1+	3+	1+	1+	4+	2+	1+	4+	2+	1+	4+	2+	2+	4+	2+	2+

Symbols 1+ to 4+ represent final degree of growth after 21 days at 37°C.

erol egg yolk medium was not satisfactory for preserving mycobacteria. However, if a protective colloid such as bovine serum albumin was used, the organisms remained viable.

On the basis of these and other observations,[3-5, 7, 9, 10] it was decided to include in this study an albumin diluent and to compare it with several other diluents such as glycerol broth, sodium chloride, charcoal water, and distilled water, all of which have been used previously for preserving mycobacteria.

The similarity of the results obtained in this investigation with each of these diluents was unexpected in view of Heckly's observations. Apparently, under the conditions herein described, the use of a protective colloid is not necessary. This further confirms the findings of Jones,[5] who obtained good recovery of various strains of mycobacteria without alteration after one year of preservation in tap water at −16° to −20° C. The observations that these mycobacteria can withstand refreezing and rethawing without significant alteration also confirm the findings of Jones.

The preliminary procedure of rapidly freezing the organisms in an ethanol-dry ice mixture, as described by Heckly[1] and Stern and Tompsett,[2] also was found unnecessary, since good results were obtained by simply placing the bacterial emulsions directly into the freezer and allowing them to freeze slowly. This technique also was employed satisfactorily by Jones.[5]

The observations on the preservation of drug-resistant human tubercle bacilli confirm those of Steenken and Wolinsky,[20] who reported that cultures of *M. tuberculosis* kept frozen for twenty-two months did not lose their resistance to streptomycin. On the basis of these findings, it is possible that other types of mycobacteria also may be preserved by freezing without significant loss in drug resistance.

The use of small, 1-dram, flat-bottom, screw-cap vials for storing the organisms is safe, convenient, economical, and requires a minimum of storage space. They have proved safer and more useful than cotton or rubber-plugged tubes. Pressure-sensitive masking tape also has proved useful in sealing the caps of the vials.

In summary: In the study described it has been found that, with several minor exceptions, various strains of mycobacteria can be preserved in several different diluents for as long as three years by freezing them at −20° C. without any significant effect on either their viability, staining, morphology, drug resistance, or their reactions to the thioglycollate, peroxidase, neutral red, serpentine cord, and niacin tests.

Although some light staining and pleomorphic bacilli were observed in the smears of the Kirchberg avian tubercle bacilli preserved in the sodium chloride, plasma, and glycerol broth diluents, most of the bacilli were identical in morphology to the control organism. Pigmentation of the photochromogenic strain AP-2 preserved in all of the diluents was more intense than the control strain. Other than these observations, no significant alterations in any of the other strains were noted.

Similar results were obtained with all of the organisms which were preserved in each diluent for as long as three years at −20° C. after they were rethawed and refrozen several times during their period of preservation.

This method of preserving mycobacteria is simple, safe, economical, and requires little material, time, equipment, and storage space.

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September 8, 1960

[20] STEENKEN, W., AND WOLINSKY, E.: Resistance of tubercle bacilli to streptomycin, in *Streptomycin, Nature and Practical Applications*, by Selman A. Waksman, Williams & Wilkins Company, Baltimore, 1949, pp. 177–196.

COMMITMENT TO A STATE MENTAL HOSPITAL FOR RECALCITRANT INFECTIOUS TUBERCULOUS PATIENTS

Compulsory isolation of recalcitrant tuberculous patients has been a controversial issue for many years. Now that we are reaching the "hard-core" cases in many areas, the tendency to enforce isolation in these cases seems to be increasing. Several states have recently put such programs into operation. Some have recently passed new laws pertaining to the subject.

Compulsory isolation of recalcitrant tuberculous patients was comprehensively discussed at the Conference on Protective Isolation in Denver in January, 1957. Stated in simple terms, the conclusions of this conference were that the problem of recalcitrant patients with infectious tuberculosis does exist; that these persons represent much more of a menace to the community than their relatively small numbers would imply; that enforced hospitalization should not be used unless every effort has been made previously to give the individual adequate care on a voluntary basis; that the facilities for regular care should be comprehensive, including medical social service and psychiatric consultation; and that the facilities for enforced isolation be well planned and staffed. The conferees re-emphasized that one of the prime requirements in handling these patients is an adequate and enlightened psychiatric staff.

This note is written to relate experiences with this problem in Denver, Colorado, in the four years since that conference. At that time, Denver General Hospital had just added two 3-bed locked wards to the tuberculosis ward; these were to be used for the enforced detention of recalcitrant infectious tuberculous patients.

A small number of "hard-core" cases were then selected; these patients were unquestionably infectious, had unquestionably been given the full benefit of existing noncompulsory facilities previously, and unquestionably were unwilling or unable to maintain themselves in isolation from other persons, especially children. Some 8 or 10 such persons received letters of quarantine signed by the City Manager of Health and Hospitals. These persons then entered the hospital, and tended in general to remain in the hospital under treatment under the quarantine orders on the open tuberculosis ward. In short, the threat of force appeared to exert a beneficial effect. A few patients deserted and one or two of these disappeared from Denver, and usually also from Colorado. Some were readmitted for further treatment. Only one person was ever detained in the locked facility, and this one only for about six weeks. He was then transferred to the open ward and later transferred to another hospital; he escaped some months later, but only after becoming noninfectious. He has not been seen since.

Early in 1958, a program of psychosocial conferences on problem patients with tuberculosis was initiated at Denver General Hospital. This was done at the instigation and with the cooperation of the Department of Psychiatry at the Hospital. In these conferences it soon became evident that these so-called recalcitrant patients were mostly sociopathic, alcoholic, and self-destructive, and gave long histories of nonconformity, unwillingness to cooperate with any authority, and a lack of normal concern for themselves and for others.

Some showed organic chronic brain syndromes, with consequent diminished judgment and inability to care adequately for themselves. Many were impulse-ridden and incapable of postponing immediate gratifications. Most of them appeared to be incapable of normal adjustment to life. Those who had been married were usually separated or divorced. They had few, if any, friends and usually no contact with members of their family.

In due course one such patient was considered to be committable by our psychiatric consultant; commitment was then accomplished through administrative channels. This patient did not react well at first but, when seen at the Colorado State Hosptial (at Pueblo) some months later, he had apparently adjusted reasonably well to this mental hospital environment. Six others have been committed in a like manner for comparable indications in the two and one-half years since. Most have tended ultimately to adjust to the mental hospital, and their tuberculosis has tended to improve significantly under these circumstances, which in essence provide enforced treatment as well as isolation.

The following two brief case histories are representative of the first patients handled in this fashion:

Case 1. G.W., a 54-year-old cook, was admitted to Denver General Hospital on a quarantine order for the seventh time on February 12, 1960, with far advanced bilateral cavitary pulmonary tuberculosis. He was first found to have tuberculosis in 1936, but had had no treatment until 1955 despite numerous pulmonary hemorrhages and repeated advice to be treated. He was first admitted to Denver General Hospital in 1955. He left without sanction after six weeks. He was readmitted for dyspnea six months later but left without sanction in ten days.

The patient spent three weeks in the hospital in 1956, five weeks in 1957, and had two other admissions in 1958 and 1959, all terminated by premature discharge. On all six admissions he was found to be infectious. He received streptomycin, PAS, and isoniazid therapy intermittently throughout these five years; he seldom received it while out of the hospital. By 1958 his organisms were resistant[1] to isoniazid, streptomycin, and PAS. The patient was adjudicated to Colorado State Hospital on March 16, 1960, with the diagnoses of passive-aggressive personality disorder and chronic alcoholism.

At Colorado State Hospital he improved considerably but was still infectious on October 1, 1960. The time and effort spent on this individual by many different professional people over the years is almost immeasurable.

Case 2. A.B., a 45-year-old laborer, was admitted to Denver General Hospital on a quarantine order on December 24, 1959, with far advanced bilateral cavitary pulmonary tuberculosis. Tuberculosis was first diagnosed in 1951. The patient spent about five of the next eight years in five different hospitals. He was discharged prematurely on five of eight different admissions. His periods in the hospital varied from one to ten months. Sputum was continuously positive for tubercle bacilli, and by 1956 the organisms were resistant to streptomycin, PAS, and isoniazid after interrupted therapy with the same three drugs. Four other members of his family have had tuberculosis; his wife had died of it. He was adjudicated to Colorado State Hospital on January 26, 1960, with a diagnosis of personality trait disorder. He improved considerably; he was then transferred to Colorado General Hospital on August 26, 1960.

Following a plombage thoracoplasty in September, 1960, sputum smears have been negative for tubercle bacilli for the first time in many years.

The psychiatric diagnoses on all 7 patients have been: personality trait disorders (sociopathic), 5; schizophrenia, 2. Four of these patients were also severe chronic alcoholics. Only 2 were married; 4 were divorced, including one female; and the other was widowed.

All 7 patients had had repeated hospitalizations and interrupted chemotherapy; all 7 had large populations of tubercle bacilli in the sputum which were predominantly resistant to one or more of the three standard drugs, streptomycin, PAS, and isoniazid; and were usually resistant to all three.

Results to date in this group have been as follows:

Escaped from Colorado State Hospital, still infectious	1
Still at Colorado State Hospital and infectious, but improved	2
Plombage thoracoplasty at Colorado General Hospital and now noninfectious	1
Lobectomy, and now discharged, noninfectious	1
Noninfectious and still at Colorado State Hospital	2
	7

It is important that the impression is not conveyed that these patients were committed for punishment purposes. It must be stressed that they lacked the adequate sense of responsibility expected from the average patient. They also have either poor reality testing, like the schizophrenics; or defective superego, like the sociopaths. As a consequence, from a psychiatric point of view, these patients not only needed external control, but such control was often very reassuring and a relief to them.

Reflecting upon the experience thus far with these few patients, it is quite apparent that a great deal of effort and time has been wasted on patients who fall into this category. These patients demand and require a great amount of effort in standard facilities, and often without much tangible benefit. Furthermore, their social behavior is often deleterious to the morale of other patients and to the efficiency of the staff in the average tuberculosis unit or hospital. In addition, the construction and maintenance of a good compulsory isolation unit which embodies proper security facilities is exceedingly costly.

Most state mental hospitals now have isolation units; today these units usually have unoccupied beds. The staffs of some of these institutions, however, now object to the admission of tuberculous patients who are sociopathic rather than overtly psychotic.

Despite this traditional objection, there seems to be no particular harm in the program as it has evolved in the Denver General Hospital; there seems to be a great deal of good in it.

A very limited experience is reported with the legal commitment to mental hospitals of tuberculous patients who are customarily regarded as candidates for the penal approach of compulsory isolation. It is suggested that others consider this

[1] Drug susceptibility is defined in this hospital as no growth on *indirect* inoculation of 3 tubes of solid 7H9 medium containing 2.0γ of streptomycin per ml., 2.0γ of PAS per ml., and 0.2γ of isoniazid per ml., respectively.

as a possible solution to the exceedingly difficult problem of the "hard-core" recalcitrant infectious tuberculous patient.

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November 7, 1960

Correspondence

L VARIATION IN MYCOBACTERIA

To the Editor of the American Review of Respiratory Diseases:

The interesting corroborative paper by Mattman, Tunstall, Mathews, and Gordon in the August issue of the REVIEW, "L Variation in Mycobacteria," mentions my own pioneer work covering a ten-year period with these forms (Ann NY Acad Sci, 1945, *46*, 127) and states that I equated "zoogleal forms" of *M. tuberculosis* with the L forms of other bacteria (p. 210), yet makes the contradictory opening remark that "The bacterial forms known as L variants have been cultured from many genera, but none have been reported as occurring in mycobacteria"!

For the benefit of those interested in carrying on further work on morphologic variations in mycobacteria, I present some additional references of work published since 1945 reporting L variations in mycobacteria *in vivo* and *in vitro*, and illustrated by photographs, electron photographs, or careful drawings. Some of these are in publications not usually read by microbiologists and, no doubt, have therefore escaped general notice.

In chronologic order they are:

GRIGORAKI, L.: Senescence et Evolution, Ch. III, Librairie Maloine, Paris, 1945.

ALEXANDER-JACKSON, E.: Non-acid-fast forms of the Mycobacterium of human leprosy, Science, 1945, *101*.

BRIEGER, E. M., AND GLAUERT, A. M.: A phase-contrast study of reproduction in mycelial strains of avian tubercle bacilli, Gen Microbiol, 1952, *7*, No. 374.

ALEXANDER-JACKSON, E.: Atypical forms of pathogenic mycobacteria, International Record of Medicine, 1954, *167*, No. 7.

XALABARDER, C.: Origin del Bacilo de Koch, Publicaciones del Instituto Antituberculoso, Barcelona, 1954.

XALABARDER, C., AND CULLEL, R.: Electron Microscopy of *My. leprae*, Publicaciones del Instituto Antituberculoso, 1955, *XI*.

WUERTHELE-CASPE, V., ALEXANDER-JACKSON, E., GREGORY, M., SMITH, L., DILLER, I. C., AND MANKOWSKI, Z.: Intracellular acid-fast microorganism isolated from two cases of hepatolenticular degeneration, J Amer Med Wom Ass, 1956, *11*, No. 4.

HÉRY, M.: Les aspects multiple des bacilles acido-resistants, Saint-Brice-s-Forêt (S et O), France, 1959.

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January 11, 1961

UNCLASSIFIED MYCOBACTERIA

To the Editor of the American Review of Respiratory Diseases:

In reference to an article "The So-called Problem of Unclassified Mycobacteria: A Review" by C. Xalabarder, in the REVIEW of January, 1961 (*83*, 1): The author of that article is incorrect in some of his references to our article, "A Limited Clinical, Pathologic and Epidemiologic Study of Patients with Pulmonary Lesions Associated with Atypical Acid-fast Bacilli in the Sputum" (Amer Rev Tuberc, 1957, *75*, 199).

On page 1, the author, in referring to our publication, states "and the affirmation that the lesions they produce in man are not tuberculous, and are therefore, of no epidemiologic significance." Our article carried no such statements. We did state, however, in our article, on page 218, "The epidemiologic findings were among the most interesting in the study. The data accumulated on family history, history of tuberculosis in the marital partner, tuberculin skin tests and roentgenograms of household contacts, all would seem to suggest that disease due to these atypical organisms is less communicable than *M. tuberculosis*."

To further substantiate our opinion at that time we now have records of more than 200 patients who have been admitted with pulmonary lesions and harboring the nonphotochromogenic strains (*Group III*, Runyon). These patients are known to have more than 600 household contacts, and we have yet to see a contact case.

On page 3 of his article the author states that we "isolated 69 unclassified mycobacteria which were designated as *yellowish* or pale orange." In our article on page 203, table 6, the 64 strains are described as "smooth buff colored." We did state on the same page, "Color varied from *buff* to light orange which became more pronounced as the cultures became older." We again stated on page 216, "Cultures from 64 of the patients in the present series were smooth, buff colored. . . ."

Dr. Ernest Runyon assisted us immensely in our early studies by identifying these 64 strains as nonphotochromogens, but all 64 strains were not sent to Dr. Runyon, as implied by the author on page 3 of his article.

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Books

HISTOPLASMOSIS. Edited by Henry C. Sweany. Pp. 538, Charles C Thomas, Springfield, Illinois, 1960. Price, $14.50.

Within the last decade, histoplasmosis, once considered an obscure and unusual infection, has been assigned its rightful place as one of the important infectious diseases of man. Its importance to the physician interested in diseases of the chest is emphasized by the fact that many patients have been kept in tuberculosis hospitals and have been treated for mycobacterial infection for years when, in fact, they were suffering from histoplasmosis.

The need for a book to summarize the available information on histoplasmosis is, therefore, obvious. When the present book was planned, it was decided that the most authoritative work could be produced by asking a group of active investigators to contribute chapters on the field of their particular interest. Thus, this volume consists of twenty-three chapters, each written by a different individual or group, with the topics selected in such a manner that the entire field is spanned. Discussion ranges from epidemiology, biology, and immunology to clinical manifestations of histoplasmosis and its pathology.

This exhaustive type of approach has its obvious advantages, but also its problems. The book will furnish the reader with the answer to any questions about histoplasmosis for which an answer is available; on the other hand, a considerable amount of overlapping of interests exists. Thus, some chapters tend to be unusually repetitious, various authors writing about the same topic in much the same way. Perhaps a clearer definition of each contributor's task and more rigid editing might have avoided this flaw.

Aside from this, however, the book is invaluable as a reference source for anyone interested in this disease. The chapters on clinical manifestations and laboratory diagnosis are unusually complete and clearly written, with much information of immediate practical value.

It should be noted that from a typographic standpoint the book is well produced, with quality paper and neatly set type. Photographs, tables, and charts are well reproduced. An author and subject index permits rapid reference to any topic of interest.

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METABOLIC CARE OF THE SURGICAL PATIENT. By Francis D. Moore. Pp. 1011, W. B. Saunders Company, Philadelphia, London, 1959. Price, $20.00.

This book is a great expansion of a somewhat similar, smaller work, *Metabolic Response to Surgery*, which Dr. Moore co-authored eight years ago. Many will welcome the substitution, as the earlier volume with its host of bar graphs raised almost as many questions as it answered. The present work answers many questions, some a bit too dogmatically, based upon work done in the surgical laboratories of the Peter Bent Brigham Hospital, and with the backing of 3,600 references. It is a guide to care, as well as a more complete description of metabolic response. Thirty-two cases are described in considerable detail to demonstrate the practice of what the book preaches and to point out wrongs of omission and commission in metabolic and fluid therapy.

The book deals primarily with body fluids, electrolytes, nutrition, and endocrines, and begins with a description of the metabolism of the normal resting patient, followed by normal convalescence from trauma and operation, and common variations of this pattern with different ages and conditions. Basic explanations of responses are given, and patterns, as the author assembles them, are lucidly described. The many parameters which can today be accurately measured are analyzed without losing sight of the fact that surgical care involves the synthesis of many factors around a sometimes unpredictable human being.

A large portion of the book deals with surgical care as it is affected by dysfunction of various organ systems. The chapters on the lung and the heart will be of special interest to readers of this journal. The author's treatment of the many fields of surgery shows remarkably accurate perception and translation.

Reportedly, the original notes for the book were "often a bit rough and uneven due to adverse air currents, roadbeds, and ocean swells." Much of the roughness and unevenness has been polished out in subsequent drafts. The writing still has some of the roll of a Sunday afternoon on Buzzards Bay, is a bit lengthy, and is nearly conversational in style, which is perhaps an autocratic but not irreproachable product from the school of the Autocrat of the Breakfast Table.

The book is nicely published and won top honors at the Fifteenth Annual Philadelphia Book Show. It will be valuable to anyone and a "Bible" to many. (We have an assistant resident, formerly

one of Dr. Moore's house officers, who almost intolerably quotes and swears by the work.) Regardless, it is a ready reference to a broad field, the most complete book on the subject available.

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ANNUAL REVIEW OF MEDICINE (Volume 11). Edited by David Rytand and William P. Creger. Pp. 453, Annual Reviews, Inc., Palo Alto, California, 1960. Price, $7.00.

The Annual Reviews have become a most important part of medical literature, and this one is no exception. As has become the practice in recent years, the papers comprising the volume are detailed accounts of current information on specific rather than general topics. This is desirable. Although specific rather than general reviews must, of course, be limited in scope, they are, as in this publication, more complete and usually more useful. The trend toward reviews of restricted topics is illustrated here by the titles of some of the papers. For example, Holman reviews "The L. E. Cell Phenomenon," while Knight reviews "Infectious Disease; Bacterial," a much more general topic. In fact, nineteen of the twenty-two subjects covered in this publication are narrow in scope, and the other three are general reviews. Of necessity, the editors of the *Annual Review of Medicine* must be able to focus upon those areas in which major advances have been made and a review is desirable and useful. Thus far, credit is due the editors for their timely selections in this volume.

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SYMPOSIUM ON PULMONARY VENTILATION. Edited by R. P. Harbord and R. Woolmer. Pp. 109, The Williams & Wilkins Co., Baltimore, 1959. Price, $4.00.

This small volume summarizes a symposium held in Leeds on February 19, 1958, under the auspices of the *British Journal of Anesthesia* and attended by several notable British physicians, physiologists, anesthesiologists, and obstetricians. In format, the book consists of seven admirably brief and concise papers on the following topics: problems of estimating alveolar carbon dioxide tension in the conscious subject and during anesthesia and artificial respiration; assistance to ventilation in the newborn; difficulties in ventilation and gas exchange during hypothermia; maintenance of ventilation in patients with chronic lung disease and carbon dioxide narcosis; mechanisms of airway obstruction; and instrumentation for measuring ventilation. The breadth of the proceedings is increased considerably by the informal, general discussion that follows each presentation. It is difficult to deduce whether the clarity, appropriateness, and intellectual restraint manifested in the discussion is due to excellent editing or to typical British performance.

In their introduction, the editors call attention to the failure of tape recordings of conferences to reveal "the mental processes involved," and express the hope that "... these, however, might be recalled by bringing the arguments into the light of print. This, when published, can provide food for thought over a much wider field." The editors have succeeded in their aim; the book is provocative and refreshing. It is worthy of perusal by all interested in pulmonary ventilation and allied problems.

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DIE STREIFENATELEKTASEN DER LUNGE. By Friedrich Heuck. Pp. 108, Georg Thieme Verlag, Stuttgart (in U. S. and Canada, Intercontinental Medical Book Corp., New York), 1959. Price, $6.70.

Dr. Heuck's monograph appeared as one of a series published by the departments of normal and pathologic anatomy of the University of Kiel. It is devoted to the problem of the disc-shaped or platelike atelectases which are not infrequently observed following abdominal disease, surgery, or trauma, and are commonly limited to the lower lobes of the lung.

The author reviewed 53,130 chest roentgenograms taken during the years 1951–1955 at the University Hospital in Kiel and found 174 cases of this type of atelectasis. He did not encounter any significant correlation with sex, age, or type of abdominal trauma. The time between appearance and disappearance of the atelectasis varied greatly. It was possible to differentiate two types: the first showed segmental distribution, while the second type was located subpleurally and transgressed the segmental borders. This second type was clearly associated with a high, fixed position of the diaphragm. The author emphasizes that the first type of atelectasis was generally thought to be caused by irritation of the thoracic nerve roots, and he gives an extensive review of the pertinent literature. In a series of ingenious experiments on anesthetized and unanesthetized guinea pigs, in

which he used electrical stimulation of selected roots of the cervical plexus and vagus nerve, he was able to produce atelectases without segmental distribution and of short duration. He further examined, through a thoracic window, the behavior of the lung after direct mechanical stimulation, and observed hyperemia associated with a decrease of the alveolar volume. From his studies he concludes that the hypothesis of a neuro-vegetative origin of the segmental platelike atelectasis must be considered unproved.

Dr. Heuck's monograph is well written and contains technically excellent photographs. His experimental method appears to be well selected and his conclusions rest on solid evidence. The scientifically inclined roentgen specialist or pathologist will find this publication of considerable interest.

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THE PRACTICAL EVALUATION OF SURGICAL HEART DISEASE. By Robert G. Trout and Robert P. Glover. Pp. 132, McGraw-Hill Book Company, Inc., 1959. Price, $10.00.

This volume is adapted from a 1957 American Medical Association convention prize-winning exhibit. It is divided into four parts. The first, on basic considerations, includes simplified physiology and sections on roentgenographic and electrocardiographic interpretations. The second part pertains to clinical studies, and presents in outline form the considerations given to the common congenital and acquired cardiac conditions for which surgery is available. Representative case presentations with accompanying roentgenographic and electrocardiographic findings illustrate the practical features for each condition. The third section, on special diagnostic techniques, briefly describes the methods of cardiac catheterization and angiocardiography. A fairly extensive bibliography is given in the final section.

This should prove an excellent book for medical students and general practitioners. There is little new to interest those who specialize in the fields of cardiology and cardiac surgery. In fact, recent advances in open heart surgery since the publication of this volume have led to new concepts in the treatment of certain forms of heart disease which, of course, could not have been included in this book. Accompanying the book is a 45-r.p.m. phonograph record on which are recorded by Dr. Scott Butterworth the heart sounds in nine different forms of heart disease.

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BOOKS RECEIVED

HAEMOPOIESIS. CELL PRODUCTION AND ITS REGULATION. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Maeve O'Connor. Pp. 490, Little, Brown and Company, Boston, 1960. Price, $11.00.

A BIOCHEMICAL APPROACH TO PATHOLOGY. By M. J. R. Dawkins and K. R. Rees. Pp. 128, Edward Arnold (Publishers) Ltd., London (The Williams & Wilkins Co., Baltimore, Maryland, U. S. agents), 1959. Price, $4.50.

LES SUPPURATIONS BRONCHIQUES, PULMONAIRES, ET PLEURALES. By Raoul Kourilsky and Guy Decroix. Pp. 259, J.-B. Bailliere et Fils, Editeurs, Paris, 1960. Price, 35 NF.

THE PHYSIOLOGICAL BASIS OF MEDICAL PRACTICE. Seventh Edition. Edited by Charles H. Best and Norman B. Taylor. Pp. 1,554, The Williams & Wilkins Co., Baltimore, Maryland, 1961. Price, $16.00.

CARDIOLOGY. AN ENCYCLOPEDIA OF THE CARDIOVASCULAR SYSTEM. Volumes 1-4. Edited by Aldo A. Luisada. The Blakiston Division, McGraw-Hill Book Company, Inc., New York, Toronto, London, 1959. Price, $100.00.

RESUSCITATION OF THE NEWBORN INFANT. Edited by Harold Abramson. Pp. 274, The C. V. Mosby Company, St. Louis, 1960. Price, $10.00.

THE METABOLIC BASIS OF INHERITED DISEASE. Edited by John B. Stanbury *et al.* Pp. 1,477, The Blakiston Division, McGraw-Hill Book Company, Inc., New York, Toronto, London, 1960. Price, $30.00.

American Thoracic Society

Medical Section of the National Tuberculosis Association

Obituaries

Gumersindo Sayago

1893–1959

Dr. Gumersindo Sayago, one of the best known and highly respected men in Argentine medicine and a leader in the battle against tuberculosis, died in Córdoba on March 29, 1959, at the age of sixty-six. For many years he had given distinguished service as a corresponding member of the American Trudeau (now Thoracic) Society.

Dr. Sayago's education was begun at the University of Buenos Aires and completed at the University of Córdoba. At Córdoba he began his academic career as instructor in pathology, later became Assistant Professor of Infectious Diseases and, still later, Professor of Phthisiology and Head of the Phthisiology Institute.

His interest in tuberculosis began quite early in his medical career, in 1919, at a time when the disease was almost completely neglected by the Argentine medical profession because of its presumed hopelessness. In 1920 he was awarded the "Alvarez Prize" for his study, "Tuberculosis in the Province of Córdoba."

The Phthisiology Institute at the Tránsito Cácares de Allende Hospital, which Dr. Sayago organized, was the major project of his life's work, and under his inspiring leadership and guidance it became an outstanding center of both undergraduate and graduate study in tuberculosis, attended by students from all of the American countries. Unfortunately, his lifelong and steadfast adherence to the ideals of democracy forced him, under political pressure, to relinquish the direction of the Institute in 1943 but, happily, he was able to return to his former post in 1955. During the interim he established a private institute in which he kept alive that spirit of scientific inquiry and service to humanity which had attracted and inspired students throughout his life.

Dr. Sayago's impatience with the delays of the printed word led him to become a vigorous traveler. In the course of his many trips abroad he maintained close personal contact and friendship with leaders in the field of tuberculosis throughout Europe and the Americas and was quick to apply, in the work of the Institute, the best of their thinking both in science and in the organization of tuberculosis control measures.

Dr. Sayago will long be remembered by his students for his kindness, patience, tolerance, and understanding of their human problems; and by his colleagues, as one of his nation's finest examples of physician, scientist, teacher, and patriot.

José Antonio Pérez

Eugenio Morelli

1881–1960

During the night of September 20–21, 1960, Eugenio Morelli—the father of modern Italian phthisiology and the teacher of many teachers in this field—passed away at the age of seventy-nine.

While still President of the National Federation Against TB, Honorary President of the Carlo Forlanini Institute in Rome, and an honorary member of the International Union Against Tuberculosis, he died on the eve of the fifteenth Italian Convention of Phthisiology. This convention had been planned with the idea of its being a personal triumph to him, and to celebrate the twenty-fifth anniversary of the founding by him of the Carlo Forlanini Institute, which he looked upon as his life's work. For this reason the convention attracted many of his disciples, including celebrated scientists from all parts of the world, and proved to be an international congress.

Eugenio Morelli was not only a famous scientist, a professor, an organizer, a soldier, and patriot but, above all, a humanitarian in the widest sense. He was a pioneer in the development of the most advanced modern methods of his day in the treatment of tuberculosis; methods which were in time adopted throughout the world.

He was one of the greatest advocates of the founding of an extensive system of scientifically up-to-date sanatoriums in connection with the Italian social security system.

In 1928 he founded the first clinic for tuberculosis and chest diseases at Rome University, and in 1935 he created the Carlo Forlanini Institute which has gained a world-wide reputation as a scientific center for study and research in tuberculosis.

Throughout his life he devoted all of his energy and time to furthering the alleviation of suffering of those afflicted with tuberculosis and chest diseases.

Countless generations of his pupils, who are now physicians and scientists holding outstanding positions in Italy and abroad, will always remember him for his benevolence and earnest desire to impart his great knowledge to others. They will continue his doctrine and his work.

MAURICIUS BLECHMANN
PASQUALINA COLANTUONO BLECHMANN

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21-25
1962—Miami Beach, Florida—May 20-24
1963—Denver, Colorado—May 12-16
1964—New York, New York—May 24-28

FOURTEENTH ANNUAL SYMPOSIUM ON PULMONARY DISEASE

The Fourteenth Annual Symposium on Pulmonary Disease will be held at Fitzsimons General Hospital, Denver, Colorado, from September 25-29, 1961. The Symposium will be co-sponsored by the American Thoracic Society, the University of Colorado School of Medicine, and Fitzsimons General Hospital.

Information concerning enrollment may be obtained from the University of Colorado Medical School or from the Program Director, Colonel Charles S. Christianson, Pulmonary Disease Service, Fitzsimons General Hospital, Denver 30, Colorado.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE
Editorial Assistant: TERRI MANN

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The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 42 abstracters systematically review 239 journals which represent 36 countries and which are published in 22 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk(*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Bilateral Multiple Lung Abscess of Hematogenous Origin: Report of a Case (in Japanese). M. Sugano*, T. Nakade, T. Kobayashi, H. Ohashi, K. Hashimoto, R. Maruya, Z. Sato, and S. Yamada. *Jap J Chest Dis*, September, 1960, 19: 645–648. (*National Otaru Sanatorium, Japan)

The lung abscess is usually solitary and is bronchogenic in origin. The writers describe a case of lung abscess which followed a periproctic furunculosis in a 20-year-old male patient. He also developed osteomyelitis of the tenth and eleventh vertebral columns. The abscesses, 15 in all, were found in all the lobes of the lungs. The patient did not have sputum production, and the blood culture was negative as he had been given antimicrobial therapy before admission. The illness was resistant to several antimicrobials, but the patient was almost cured after he had been given 13 gm. of chloramphenicol, 1.23 million units of penicillin, 8 gm. of streptomycin, 31.3 gm. of oxytetracycline, 61 gm. of tetracycline, and 75 gm. of erythromycin. All of the cavities disappeared 5 months after their development.—I. Tateno

Idiopathic Acquired Agammaglobulinemia Associated with Thymoma: Report of Two Cases and Review of the Literature. J. Gafni*, D. Michaeli, and H. Heller. *New Engl J Med*, September 15, 1960, 263: 536–541. (*Tel-Hashomer Hosp., Tel-Aviv, Israel)

Two cases of idiopathic acquired agammaglobulinemia associated with thymoma are reported, bringing the total of reported cases to 6. The 4 such cases reported in the literature are summarized. It is clear that the association of thymoma and agammaglobulinemia cannot be ascribed to chance alone, as it has now been reported in 6 of 46 cases of idiopathic acquired agammaglobulinemia. This figure approaches the 15 per cent incidence of thymoma in myasthenia gravis. If a thymoma is looked for in every case of acquired agammaglobulinemia and if electrophoretic studies are performed in every case of thymoma, many more cases may well be discovered. This is especially true in view of the fact that, in 3 of the patients, the presence of the mediastinal mass was known before the appearance of a clinical picture that could arouse suspicion of agammaglobulinemia, and in 2 patients, recurrent infections were not at all prominent. It would be of interest to follow the known cases of acquired idiopathic agammaglobulinemia to learn the weight and histologic structure of the thymus when no tumor is clinically apparent. Inability to explain the pathophysiologic mechanism underlying the association of thymoma and agammaglobulinemia is but a reflection of a lack of basic knowledge of normal thymic function. Medicine is still at the stage of collecting clinical observations of the association of thymic hyperplasia or tumor with conditions such as myasthenia gravis, aplastic anemia, and Cushing's syndrome. Idiopathic acquired agammaglobulinemia must be added to this list.
—M. J. Small

Fibrinoid Necrosis in the Branches of the Pulmonary Artery in Chronic Non-Specific Lung Disease. M. S. Dunnill*. *Brit J Dis Chest*, October, 1960, 54: 355–358. (*Radcliffe Infirmary, Oxford, England)

In recent years there have been numerous reports of fibrinoid necrosis and arteritis affecting the branches of the pulmonary artery in primary pulmonary hypertension and in pulmonary hypertension complicating congenital or acquired heart disease. The present case is reported because it occurred in a man with chronic bronchitis and emphysema who was dying of right heart failure. The muscular pulmonary arteries showed a striking degree of fibrinoid necrosis. In this case, it is probable that the chronic bronchial and bronchiolar infection, with consequent anoxia, was never adequately controlled. The anoxia and raised pulmonary artery pressure were thus maintained for an unusually long period, some 6 months, and this was probably the most important factor causing the arterial lesions.—M. J. Small

Pneumopathy during Rheumatoid Arthritis (in French). E. C. Bonard* and H. Vasey. *Schweiz Med Wschr*, August 13, 1960, 90: 866–870. (*Policlinique Universitaire, Geneva, Switzerland)

Eighteen of 50 cases of rheumatoid arthritis showed evident roentgenographic pulmonary lesions. In 19 cases, the clinical examination revealed some respiratory disorder. Although it is impossible without a biopsy to prove the rheumatoid origin in every case, from the high incidence of the coexistent pulmonary lesions it can be concluded that some relation must exist between the pneumopathies and the arthropathy.—J. Haapanen

Sarcoid Arthritis with a Response to Colchicine: Report of Two Cases. H. Kaplan*. *New Engl J Med*, October 20, 1960, 263: 778–781. (*U. S. Army Hosp., Munich, Germany)

The diagnosis of sarcoidosis was established histologically in two young Negro patients. Both had characteristic hilar adenopathy as judged by chest roentgenograms. One patient had recurrent polyarthritis that responded dramatically to colchicine on 4 occasions. Four untreated attacks of

joint pain persisted for 10, 14, 5, and 6 days, respectively. In the second patient, a course of colchicine terminated a 2-month episode of bilateral knee effusion. Evidence is presented to distinguish the arthritis in such cases from primary gout, although a coincidence of gout and sarcoidosis cannot be definitely excluded. This represents the first report of the effectiveness of this ancient remedy in arthritic conditions other than gout. On the basis of these observations, speculations are offered about a possible interrelationship between sarcoidosis and gout.—M. J. SMALL

Prolonged Steroid Therapy in Childhood Asthma. J. P. ANDERSON*. *AMA J Dis Child*, September, 1960, 100: 341–352. (*Monsall Hosp., Manchester, England)

Thirty-nine children with moderate or severe chronic asthma unresponsive to routine treatment were given oral prednisolone for a minimum of 12 months initially. The responses, when assessed in September, 1957, were "good" or "excellent" in 31 and "fair" in 7 cases. One boy died after 8 months from unsuspected pneumonia, septicemia, and adrenal failure. One girl developed acute adrenal failure from an undertreated respiratory infection after 15 months; she recovered with treatment. Respiratory infections were the only common complications; they responded to antimicrobial treatment, but caused some temporary return of bronchospasm. Side effects were unimportant, except for insidious growth suppression in 7 children which was long overlooked. From October, 1957, attempts were made to withdraw steroid treatment in the 38 survivors. The initial severity of the child's asthma was the decisive factor in attempting to discontinue regular steroid therapy; this proved impossible in 11 of 13 severe cases, but in only 8 of 25 moderately severe cases. It is concluded that the benefits of long-term steroid therapy can outweigh the dangers in certain carefully selected cases of severe chronic childhood asthma when routine measures have proved consistently ineffective. Close supervision by experienced observers is essential.—M. J. SMALL

Broncholithiasis Terminating in Death Due to Massive Hemoptysis (in Japanese). K. SAKAKI*, N. SOMA, and T. HARADA. *Jap J Chest Dis*, October, 1960, 19: 720–722. (*Aomori Central Hosp., Japan)

The authors describe the case of a 33-year-old male patient whose chief complaint was recurrent hemoptysis. Bronchography revealed a defect in the right lower bronchial shadow, and bronchoscopy revealed a black-brown foreign body with a rough surface on the lateral wall of the middle portion of the right lower bronchus. The foreign body was easily movable with a forceps and was carefully removed. A granulation tissue remained behind and there was no hemoptysis following operation. The second bronchoscopy was performed 3 months after the patient had an uneventful recovery. The findings at examination were a smaller granulation tissue than that observed at the first examination, and a black mass covering the center of the granulation. Careful attempts to remove this tissue were unsuccessful. On the third day following examination, the patient expectorated 10 and 15 ml. of blood, which increased the next day, when 1,600 ml. of blood were expectorated. The patient died of suffocation due to massive hemoptysis on the sixth day as he was being carried into an operating theater. The authors recommend early thoracic surgical intervention when an intramural broncholith is found or expectorated spontaneously.—I. TATENO

Closed-Chest Cardiac Massage. W. B. KOUWENHOVEN*, J. R. JUDE, and G. G. KNICKERBOCKER. *JAMA*, July 9, 1960, 173: 1064–1067. (*Johns Hopkins U., Baltimore, Md.)

As a result of animal experimentation, a method of external transthoracic cardiac massage has been developed which can easily be applied by a lay person. With the patient in supine position, preferably on a rigid support, the heel of one hand with the other hand on top of it is placed on the sternum just above the xyphoid. Firm pressure is applied vertically downward about 60 times per minute. Sufficient pressure should be used to move the sternum 3 or 4 cm. toward the vertebral column. At the end of each stroke, the hands are lifted slightly to permit full expansion of the chest.—H. ABELES

Chylothorax: Review of 5 Years' Cases in the Literature and Report of a Case. A. SCHMIDT*. *Acta Chir Scand*, November, 1959, 118: 5–12. (*Copenhagen, Denmark)

A total of 92 cases of chylothorax were collected. One-third of the cases occurred spontaneously. In adults, the most frequent trauma was pulmonary operation for tuberculosis. Conservative treatment was used in 50 cases and resulted in 36 cures and 11 deaths; the deaths were directly or indirectly referable to the chylothorax. It is concluded that present-day operative treatment involves only minimal risk, but that conservative therapy offers better prognosis than previously assumed, especially in children.—R. MACQUIGG

Coccidioidomycosis. The Need for Careful Evaluation of the Clinical Pattern and Anatomical Lesions. W. A. WINN*. *Arch Intern Med*, October, 1960, 106: 463–466. (*Tulare-

Kings Counties Hosp., Springville, Calif.)

In a disease of such variation as coccidioidomycosis, it is only by long-continued study that basically sound conclusions may be drawn with regard to the clinical patterns, anatomic lesions, and pulmonary roentgenographic changes. Proper clinical management of coccidioidomycosis requires careful and frequent roentgenographic and serologic study of the patient from the very first phase of the primary infection. If dissemination threatens, as manifested by a rising titer of complement fixation, and there is continued illness of the patient, the intravenous use of amphotericin B may well be indicated. If residual pulmonary foci form, their careful roentgenographic and serologic appraisal is indicated. The coccidioidoma will require removal when diagnosis is not certain and malignancy is possible. The abscessing coccidioidoma will usually require surgical excision. The residual cavity will require removal if recurrent hemoptysis is present, if it is more than 2 cm. in diameter and has persisted for 1 year, if it is subpleural and rupture threatens, if it is enlarging, and if secondary infection has occurred within the cavity with resultant "ballooning" and failure to respond to antibacterial therapy. Preoperative and postoperative coverage with amphotericin B should be considered for excisional therapy of pulmonary cavities, infected bone, gonads, lymph nodes, and peripheral granulomas. Even with such coverage, excision should be wide enough to avoid transection of infected tissue. Careful medical supervision and definitive surgical and antifungal treatment will control the local extension of abscessing coccidioidomas, the enlargement and transpleural rupture of cavities, and the appearance of recurrent cavitation following resection. The residual pulmonary lesion that shows evidence of local activity, or even progression, very rarely produces dissemination of coccidioidal disease. When it occurs, dissemination may be slow but is usually rapid.—W. J. Steininger

Prognosis of the Eczema-Asthma Syndrome. D. Burrows* and R. W. B. Penman. *Brit Med J*, September 17, 1960, No. 5202: 825–828. (*Royal Victoria Hosp., Belfast, Ireland)

The results are reported of a review, 9 to 12 years later, of 43 children who were originally admitted to a skin unit with eczema; 38 were traced and investigated. Of the 29 cases of true infantile eczema, 24 (82.8 per cent) still had eczema. Of 20 children investigated from the respiratory viewpoint, 16 (80 per cent) had chest symptoms. Only 10 per cent of the 38 children were free of both skin and chest complaints. Some aspects of the prognosis of the eczema-asthma syndrome are discussed, with some observations on the use of tests of ventilatory function in the assessment of these children (after authors' summary).—E. A. Riley

Congenital Lobar Emphysema. H. A. Myers*. *Aust NZ J Surg*, August, 1960, 30: 32–35. (*Royal Children's Hosp., Melbourne, Australia)

Cases of congenital lobar emphysema are described. Attention is drawn to the fact that this condition can be recognized in 3 clinical groups, namely, in infancy, in older children with, and also in older children without symptoms. Although no common etiologic factor has as yet been demonstrated, an occasional cartilage deficiency in the bronchi has been reported. The causes of breathlessness in babies are discussed, and it is emphasized that they may be, and often are, due to a condition remediable by surgical means.
—R. M. Ford

Interstitial Pulmonary Fibrosis Secondary to Pulmonary Venous Hypertension—Report of a Case Due to Myxoma of the Left Atrium. M. Solomon*. *JAMA*, October 1, 1960, 174: 464–467. (*Charity Hosp., New Orleans, La.)

A 29-year-old housewife complained of increasing dyspnea, cough, and wheezing. A murmur characteristic of mitral stenosis was heard. The chest roentgenogram showed diffuse interstitial infiltration and prominent pulmonary conus. A lung biopsy revealed granulomatous infiltration. Pulmonary function studies suggested interstitial fibrosis, and catheterization of the right heart demonstrated pulmonary vascular hypertension. In the course of a mitral commissurotomy, a left atrial mass was found which was subsequently removed. The histologic report was myxoma of the heart with adenomatoid configuration. Six months after operation, the respiratory symptoms and the alveolar capillary block disappeared, but the interstitial fibrosis persisted.—H. Abeles

A Case of Multiple Pulmonary "Hamartomata" of Unusual Type. R. Y. Keers* and F. A. Smith. *Brit J Dis Chest*, October, 1960, 54: 349–352. (*Chest Clinic, Stoke-on-Trent, England)

A case of multiple benign bilateral pulmonary tumors is described in which the histologic pattern was predominantly fibroadenomatous. Nomenclature is discussed on the basis of the original definition of hamartoma, and the descriptive title "benign pulmonary fibroadenomatosis" is suggested. This case is reported in detail because the authors have been unable to find any record in the literature of an instance of multiple pulmonary lesions of similar type, and also because it provides an exception to the general rule that benign tumors and developmental abnormalities in the lung occur singly. In describing it as an instance

of multiple hamartomas, the term "hamartoma" is being used in its broadest sense.—M. J. SMALL

Histiocytosis X. Report of a Case of Hand-Schüller-Christian Disease. A. E. GREEN, JR.* and R. A. FLAHERTY. *Radiology*, October, 1960, 75: 572–576. (*Lackland Air Force Base, Texas)

A case is reported of Hand-Schüller-Christian disease, one of the entities included under the more general designation of histiocytosis X or reticuloendotheliosis. The patient, a white female 18 months old, had extensive somatic involvement with remission following supportive care and roentgen therapy for bone and lung lesions. Noteworthy results were the reversal of pulmonary manifestations (interstitial inflammation and fibrosis), the response to therapy of severe anemia, and the appearance of normal new bone without osteolytic defects at the epiphyses soon after clinical remission. The latter may be a useful prognostic sign for evaluating similar cases.
—W. J. STEININGER

On an Unusual Case of Bronchial Leiomyoma (in Japanese). K. KUDO*, Y. WATARI, and H. HATSUKANO. *Jap J Chest Dis*, October, 1960, 19: 723–727. (*Kekkaku Yobokai Institute, Kiyose, Japan)

The authors describe a case of benign leiomyoma of the left main bronchus in a 19-year-old male student. The patient was initially seen with the complaint of fever and cough. Roentgenographic examination 7 months before operation revealed the trachea displaced toward the left side of vertebra, and both the heart and atelectatic left lung were displaced in the left hemithorax. Bronchography revealed stenosis of the left main bronchus just below the bifurcation. Both lobes of the left lung were atelectatic and atrophic at operation, and pneumonectomy was performed. The tumor was a round mass with a diameter of 1.5 cm., and was diagnosed histologically as benign leiomyoma. A review of the literature revealed that 12 cases of bronchial leiomyoma had been reported, including the present case. One case was described as fibroleiomyoma. The age of the patients ranged from 8 to 63 years, and there were 5 cases in males and 7 in females. The tumor originated from the bronchus in 6 cases, from the lung parenchyma in 2 cases, and was not described in 4 cases.
—I. TATENO

Differential Diagnosis of Chronic Pulmonary Processes (in German). J. GARTMANN*. *Schweiz Z Tuberk*, No. 3, 1960, 17: 161–178. (*Kantonal-Zürcherische Heilstätte Altein, Arosa, Switzerland)

The case histories are presented of 5 patients with various chronic pulmonary diseases, and the difficulties in establishing the diagnosis are discussed. Three patients had cavitary processes, 1 caused by tuberculosis, another by suppurative pneumonitis, and the third by bronchogenic carcinoma. In 2 other patients, positive acid-fast bacillary findings made the diagnosis of pulmonary malignancy difficult, but it was later proved by thoracotomy.—Z. VIRÁGH

Mediastinal Tumors and Cysts in the Adult. M. JOANNIDES, JR., and H. JANGSTON*. *Dis Chest*, September, 1960, 38: 243–249. (V. A. Hosp., Hines, Ill.)

Statistically, this series of 117 patients with proved mediastinal tumors or cysts cannot be compared to other reported series because there is only 1 woman in the group, and because the age group (21 to 70 years) is quite selective. Thirty-eight different histologic types were seen. A specific preoperative diagnosis is unlikely, and early thoracotomy is advisable. The routine preoperative utilization of scalene node biopsy and the more frequent use of angiocardiography are particularly stressed.—E. A. ROUFF

Localized Mesotheliomas of the Pleura: The Pathologic Evaluation of 18 Cases. E. A. FOSTER* and L. V. ACKERMAN. *Amer J Clin Path*, October, 1960, 34: 349–364. (*Washington U. School of Med., St. Louis, Mo.)

Of 18 cases of localized pleural tumors, most occurred in the sixth and seventh decades of life, but the youngest patient was 17 years of age when his tumor was discovered. There was no predilection for either of the sexes. Most of the tumors grew slowly and produced few symptoms. The tumors were variously attached to the parietal or visceral pleura, lay in an interlobar fissure, or were buried in the lung. Three histologic varieties of tumor were observed: epithelial (papillary), fibrous, and mixed. All of the tumors were histologically benign and there have been no proved recurrences or metastases. The possibility that some of the tumors were malignant, however, was not entirely excluded. The findings added support to the thesis that all 3 histologic types were derived from the pleural mesothelium and that the classification of the tumors as mesotheliomas was justified.—E. DUNNER

Electrokymographic Changes in Myocardial Infarction. O. BARTLEY*. *Acta Radiol (Stockh)*, July, 1960, 54: 81–89. (*Sahlgrenska Sjukhuset, Gothenburg, Sweden)

This report concerns electrokymographic changes observed in the movements of the ventricles, atria, and great vessels in a series of verified myocardial infarctions. A direct recording electrokymograph

linked with a heart tone attachment was used, as well as electrocardiograms and phonocardiography. Thirty-six patients were studied. The changes observed occurred in systole or diastole, or during both. During systole, a localized reduction or absence of movement was noted in the ejection phase; a completely or partially paradoxical movement could also be noted. In the atria, a pathologic auricular contraction was noted. This means that a contraction occurred in which the amplitude exceeded that of the rapid filling phase. Abnormal movements with a late and slow upstroke and a high, often-obliterated incisura were seen in the pulmonary artery curves. Another movement had the appearance of a conspicuously rapid collapse of the vessel immediately following the dicrotic wave.—E. Shabart

Periosteal Chest Wall Pain as Part of the Post-Myocardial-Infarction Syndrome. H. Roesler*. *Amer J Med Sci*, October, 1960, 240: 407–410. (*Temple U. Med. Center, Philadelphia, Penn.)

In 2 cases of recent myocardial infarction, evidence was found for periosteal involvement of chest wall structures, giving rise to pain that was abolished by the local injection of a corticosteroid. This periosteal involvement is thought to be of an allergic-inflammatory origin and represents an addition to the triad of pneumonitis, pleurisy, and pericarditis that characterizes Dressler's post-myocardial infarction syndrome (Author's summary).—W. J. Steininger

Pulmonary Manifestations of Pancreatitis. D. M. Roseman*, O. D. Kowlessar, and M. H. Sleisenger. *New Engl J Med*, August 11, 1960, 263: 294–296. (*New York Hosp., New York)

This report concerns a housewife with recurrent pancreatitis in whom the complications of mediastinal abscess, pulmonary infiltrates, and pleural effusion obscured the underlying pancreatic inflammation for a long while. Multiple intra-abdominal abscesses communicating to the mediastinum were eventually found at operation. In a study of 228 cases of proved pancreatitis at the New York Hospital, pulmonary abnormalities were detected in 20 per cent. Elevation of the diaphragm, pleural reaction, pleural effusion, lung infiltration, and basilar atelectasis were the signs noted on the initial roentgenographic study. A careful roentgenographic study of the lung fields should be done in all patients with suspected pancreatitis. Conversely, pancreatitis should be considered in the differential diagnosis of any patient who presents unexplained alterations in the right or left basilar lung fields. When pleural effusion is present, its amylase content should be compared with that of a simultaneous serum specimen; markedly elevated pleural fluid amylase values as compared to serum values have been reported in pancreatitis, whereas in pleural effusion associated with nonpancreatic disease, the pleural fluid amylase is usually less than the simultaneous serum value.—M. J. Small

Needle Biopsy of the Pleura in the Differential Diagnosis of Exudative Pleurisy (in German). M. Toušek* and J. Dûra. *Schweiz Med Wschr*, July 16, 1960, 90: 782–785. *(Bezirkskrankenhaus, Nový Bydžov, Czechoslovakia)

Pleural biopsy with a Vim-Silverman needle was performed on 60 patients with exudative pleurisy. In 33 cases a definite diagnosis was obtained: in 16 cases, tuberculous pleurisy; in 12, malignancy; in 3, nonspecific empyema; in 1, lupus erythematosus disseminatus; and in 1, malignant lymphogranuloma. Before the biopsy was done, 18 of these cases were labeled "idiopathic exudative pleurisy." In 15 cases the histologic examination showed nonspecific inflammatory changes. In 10 of these cases the finding supported the clinical diagnosis (post-traumatic pleurisy, metapneumonic pleurisy, or pleurisy after pulmonary infarction). Thus, in almost ¾ of all cases the diagnosis was either made or supported by the biopsy. In 9 cases the specimen did not reveal any characteristic changes. In 3 cases the result was false negative; in 2 of these the diagnosis of a malignant tumor was made later, and 1 case proved to be tuberculous.—J. Haapanen

Acquired Communication Between the Pleural Cavities: Report of a Case. E. J. M. Weaver*. *Brit J Dis Chest*, October, 1960, 54: 353–354. (*London Hosp., England)

Communication between the pleural cavities is not a previously recognized abnormality. Such a case is reported in a man who had been receiving bilateral pneumothorax for pulmonary tuberculosis for 26 years until 1946. No further treatment was given until 1951 when, owing to breathlessness, monthly right pleural aspirations were begun at a chest clinic. In 1954, while an aspiration was being performed, the needle broke in the right pleural cavity. At that time, it was decided that no attempt should be made to remove it, but that the patient should attend the clinic and that his progress should be assessed regularly. A chest roentgenogram taken in 1955 showed that the needle was now lying in the left hemithorax, but one taken a month later revealed that it had returned to the right pleural cavity. A pleurogram demonstrated a communication between the pleural cavities. At thoracotomy, the needle was removed and the communication leading to the left pleural space was seen to be a circular opening about 2

cm. in diameter, situated in the superior mediastinum, lying immediately posterior to the upper end of the sternum.—M. J. Small

Fatal Pneumocystic Pneumonia in an Adult: Report of a Case. C. D. Anderson* and H. J. Barrie. *Amer J Clin Path*, October, 1960, 34: 365–370. (*U. of Toronto, Canada)

Necropsy examination of a 36-year-old man revealed that he had had almost solid filling of the lung with *Pneumocystis carinii*. The patient had been ill for 10 months with increasingly frequent attacks of fever, chills, and cough, and had had no intercurrent disease except for a slight degree of cirrhosis. Microscopic examination of the lungs revealed little reaction other than severe edema, swelling, and separation of the components of the alveolar walls. Only a few organisms were observed in the cytoplasm of histiocytes in the lung and in the sinuses of a hilar lymph node. Review of material collected during antemortem bronchoscopy revealed recognizable parasites (after authors' summary).—E. Dunner

Pulmonary Alveolar Proteinosis with Systemic Nocardiosis. B. R. Andersen*, R. E. Ecklund, and W. F. Kellow. *JAMA*, September 3, 1960, 174: 28–31. (*U. of Illinois, Chicago)

A case of alveolar proteinosis is reported which was proved by lung biopsy and in which disseminated nocardiosis developed. Although the fungous infection responded well to specific treatment, the pulmonary changes due to the original disease remained unchanged during the 1-year follow-up period.—H. Abeles

Lung Biopsy in Sarcoidosis: With Special Reference to Bacteriological and Microscopic Features. L. Reid* and G. Lorriman. *Brit J Dis Chest*, October, 1960, 54: 321–334. (*Brompton Hosp., London, England)

The clinical, roentgenographic, and pathologic findings are reported in 16 cases of sarcoidosis diagnosed by means of lung biopsy. In 1 patient there was strong evidence of a tuberculous etiology. The lung biopsy specimen yielded *M. tuberculosis* on culture and gave a positive guinea pig inoculation test although the patient was Mantoux negative. Necrosis resembling caseation was found in hilar nodes excised from 2 patients. Caseation was nonetheless excluded by the use of stains for collagen and reticulum. These stains, Verhoeff's elastic counterstained with Van Gieson, and Gomori's reticulin stain, should always be done to differentiate the "granular necrosis" which occurs fairly often in sarcoidosis from "caseation necrosis" which implies a diagnosis of tuberculosis. In 1 patient the pathologic changes preceded roentgenographic abnormalities by several months. The findings in this series suggest that lung biopsy with simultaneous excision of a hilar node will provide a positive diagnosis in practically all patients with sarcoidosis and lung involvement. In particular, lung biopsy is more likely to be successful than liver biopsy in those patients whose chest roentgenograms show diffuse pulmonary infiltration without evidence of hilar node enlargement.—M. J. Small

Pectus Carinatum, Pigeon Breast and Related Deformities of the Sternum and Costal Cartilages. C. W. Lester*. *Arch Pediat*, October, 1960, 77: 399–405. (*Hosp. for Special Surgery, New York)

Pectus carinatum is a protrusion deformity of the sternum and costal cartilages developing with growth. It may be in the midline or lateral to the sternum or in the costal arch. Symptoms can be both physical and psychologic. The respiratory symptoms in pectus carinatum are those of an ineffectual cough. Because the bronchial secretions cannot be expelled, a mild bronchitis becomes chronic or progresses to pnemonitis. The treatment is surgical and the results are good.—M. J. Small

The Thymus and Myasthenia Gravis. Editorial. *New Engl J Med*, September 1, 1960, 263: 467.

Although the nature of the physiologic action of the thymus is not fully understood, much has been added in recent years to our knowledge of the pathology of the gland and to a visualization of the clinical relation existing between this structure and myasthenia gravis. Castleman has shown that nearly 80 per cent of the patients with myasthenia also show some changes in the thymus, and about 15 per cent will have thymomas, usually considered a benign type of tumor but often spreading locally without distant metastases. All patients with thymoma, however, may not show signs of myasthenia gravis. The picture is quite the opposite in patients with myasthenia gravis but without thymoma, particularly in the female group. Continuing reports reinforce the concept that females less than 35 years of age with myasthenia gravis who do not have a thymoma respond to thymectomy and, indeed, may live for many years without symptoms of the disease. Current findings indicate the favorable effects of thymectomy on patients with myasthenia gravis, but even more strongly the unfavorable results in patients with thymoma whether or not they have signs of myasthenia. It is possible that radiation therapy is the best means of treating thymomas in spite of the fact that removal would seem to be indicated. Gradually, a clear idea of the thymus and its functions is being disclosed. —M. J. Small

Primary Pulmonary Torulosis. Z. VIRÁGH*. *Schweiz Z Tuberk*, No. 3, 1960, 17: 186–195. (*V. A. Hosp., Outwood, Ky.)

The present status of pulmonary torulosis (cryptococcosis) is discussed in association with a case observed and documented with serial roentgenograms. The diagnosis, ascertained by animal inoculation of the fungus cultured from the patient's bronchial secretions, was established only after the patient left the hospital as cured (after author's summary).

Wegener's Granulomatosis with Discrete Lung Lesions and Peripheral Neuritis. D. J. MACFADYEN*. *Canad Med Ass J*, October 1, 1960, 83: 760–764. (*University Hosp., Saskatoon, Canada)

A case of Wegener's granulomatosis is described in which the clinical diagnosis was confirmed at autopsy. The relationship of this disease to other vasculitides is discussed. The metastatic-like, granulomatous lung lesions, as well as evidence suggestive of hypersensitivity, symptomatology, and nervous system lesions are also discussed (after author's summary).—E. A. RILEY

TUBERCULOSIS, PULMONARY

Intrapleural Hydrocortisone in Tuberculous Pleural Effusion. K. S. MATHUR*, R. PRASAD, and J. S. MATHUR. *Tubercle*, October, 1960, 41: 358–362. (*Sarojini Naidu Med. College, Agra, India)

Fifty patients with tuberculous pleural effusions were studied. The diagnoses were based on clinical evidence, routine laboratory investigations, and pleural biopsies. Twenty-five patients who served as controls were given streptomycin and isoniazid. In the remaining 25, pleural biopsy was done and at the same sitting hydrocortisone was injected intrapleurally—250 mg. in the first 10 patients and 125 mg. in the remaining 15. Therapeutic aspiration was not done even when the effusion was massive. In cases in which fluid persisted, second or third biopsies of the parietal pleura were done and the same dose of intrapleural hydrocortisone was repeated every fortnight up to a maximum of 4 instillations. The 2 groups were comparable in factors likely to affect the response to antituberculous treatment alone. The response to therapy was "striking" (absorption within 15 days) in 18 cases, "good" (absorption within 30 days) in 3, "fair" (absorption within 60 days) in 2, and "unsatisfactory" (fluid persisted after 60 days) in 2 of the 25 cases treated with intrapleural hydrocortisone. In the control group the response was "striking" in none, "good" in 2, "fair" in 7, and "unsatisfactory" in 16 cases. The residual pleural thickening was also less in the treated group.—M. J. SMALL

Bacteriologic Mechanism and Clinical Application of Alternating Therapy in Pulmonary Tuberculosis (in Japanese). K. URUSHIZAKI*. *Clin Resp Organs*, September, 1960, 15: 611–618. (*National Tokyo Sanatorium, Kiyose, Japan)

The writer alternated antimicrobial therapy by employing: (*1*) streptomycin and PAS for one period and pyrazinamide and isoniazid for another period every 3 or 6 months, or (*2*) kanamycin, cycloserine, and PAS for one period and sulfisoxazole, pyrazinamide, and isoniazid for another period every 3 or 6 months, respectively, in 115 patients with pulmonary tuberculosis who were being re-treated with isoniazid. Serial peroxidase tests were also made on tubercle bacilli isolated from the sputum. It was found that, when almost all of the colonies of tubercle bacilli isolated from the patient became peroxidase negative, it was impossible to make the sputum culture negative for the bacilli with any form of therapy. The aim of the chemotherapy was, therefore, to eradicate the bacilli when there were mixed populations of peroxidase-positive and -negative colonies. The results of treatment during this phase with a combination of streptomycin-PAS-isoniazid brought about eradication of tubercle bacilli in 50 per cent of the cases. The goals of chemotherapy in pulmonary tuberculosis should be: (*1*) to avoid making all of the bacilli populations peroxidase negative and isoniazid resistant, and (*2*) to make the bacilli susceptible to isoniazid by an artificial method (for instance, withdrawal of chemotherapeutics, et cetera), and employ intensive isoniazid therapy alternately when retreatment with drugs is needed. The time of changeover from one form of therapy to another may be 2 weeks for the previously untreated cases and 4 weeks to 1 month for the re-treatment cases. —I. TATENO

Cavitating Primary Pulmonary Tuberculosis in Infancy. N. JOFFE*. *Brit J Radiol*, October, 1960, 33: 430–439. (*Baragwanath Hosp., Johannesburg, South Africa)

Twenty-seven African infants who had cavitating primary pulmonary tuberculosis during the first 2 years of life were studied. The youngest infant was 6 weeks old, but the peak incidence occurred at ages 7 to 9 months and 20 to 24 months. There were 16 males and 11 females. The commonest symptom was cough, but fever, irritability, vomiting, and diarrhea were often noted. Primary cavitation is usually due to a breakdown occurring

within a progressive primary focus. The cavities usually had irregular ragged walls, and were most often found in the right upper lobe, right middle lobe, or left upper lobe. Cavitation involving more than 1 lobe was noted in 2 cases. Miliary tuberculosis was also present in 3 of the 27 cases. Differential diagnosis must also include suppurative pneumonia and lung abscesses.—L. HYDE

Is Cavity Closure in Pulmonary Tuberculosis Influenced by Bed Rest? N. WYNN-WILLIAMS* and J. B. SHAW. *Tubercle*, October, 1960, 41: 352–357. (*St. Mary's Hosp., Luton, England)

A group of 62 patients with tuberculous cavitation caused by drug-susceptible organisms was treated with antituberculosis drugs and allocated by random selection to regimens of bed rest or exercise until cavity closure. In the rest regimen, the patients remained in bed except for toilet purposes. Patients allocated to the exercise regimen remained in bed only if they felt ill, or were febrile. As soon as ill patients felt well enough to get up, they were allowed to do as they pleased, except that they were discouraged from taking exercise which made them breathless or fatigued. The condition of each group of patients was assessed at 6 months. No difference was found between the 2 groups in the time taken for cavity closure, sputum conversion, and reduction of the erythrocyte sedimentation rate. Further assessment after 12 months showed similar results between the 2 groups in the time taken for cavity were away from work more than twice as long as those in the "exercise" group. Patients should not be put to bed for long periods of time until proof of the value of bed rest in treatment is obtained. —M. J. SMALL

TUBERCULOSIS, NONPULMONARY

The Diagnosis and Prognosis of Tuberculous Meningitis (in German). H. JOOS* and W. PULVER. *Schweiz Med Wschr*, August 6, 1960, 90: 842–850. (*Kantonsspital, Lucerne, Switzerland)

In a series of 68 cases of tuberculous meningitis, concurrent tuberculous lesions were found in 97 per cent. Tuberculin tests were positive in 93 per cent; 31 per cent had been treated previously for tuberculosis, and in 12 per cent the source of infection was evident. None had been vaccinated with BCG. The pretreatment findings in the cerebrospinal fluid were: leukocytosis in 100 per cent, high globulin content in 98.5 per cent, decreased sugar in 78 per cent, low chloride in 74 per cent, coagulation on standing in 66 per cent, and the discovery of tubercle bacilli by microscopy or culture in 66 per cent. The prognosis has been much more favorable since the discovery of isoniazid as an antituberculosis agent. The prognosis depends mostly on the general condition at the time therapy is started.—J. HAAPANEN

Tuberculous Osteomyelitis of the Clavicle, A Report of Five Cases. S. ROSENDAHL-JENSEN*. *Acta Chir Scand*, February, 1960, 118: 327–330. (*Nationalforeningens Hosp., Juelsminde, Denmark)

Skeletal tuberculosis involves the spine in approximately ½ of all cases, and the smaller bones are less frequently implicated, the chief sites being the wrist, greater trochanter of the femur, and pubic symphysis. Tuberculosis of the clavicle is uncommon. Some investigators question its existence, and its rare occurrence is surprising in view of the relative frequency with which that bone is affected by chronic diseases such as syphilis. The present paper reports 5 cases diagnosed in 4 years; in 3 the medial, and in 2 the lateral ends of the bone were implicated. Treatment consisted of a combination of surgical and antimicrobial methods. On follow-up, all patients were found to be free of symptoms, and no recurrence was demonstrable roentgenographically.—R. MACQUIGG

Cortisone and Skin Sensitivity to Tuberculin in Reticuloses. G. H. FAIRLEY* and J. Q. MATTHIAS. *Brit Med J*, August 6, 1960, No. 516: 433–436. (*St. Bartholomew's Hosp., London, England)

Skin sensitivity to tuberculin with and without local cortisone was determined in 138 patients with reticuloses, 43 patients with carcinoma, and 116 controls. The previous reports that tuberculin sensitivity is depressed in reticuloses have been confirmed. In this study, 45 per cent of the patients with these diseases were insensitive, compared with 18 per cent of those with carcinoma and 12 per cent of the controls. The possible reasons for this are discussed. In the patients in all 3 groups who responded to 10 or 100 TU, cortisone tended to reduce the size of the reactions, an effect which was more marked with 100 than with 10 TU. In the patients who were insensitive to tuberculin, 8 per cent of those with reticuloses, 7 per cent of the controls, and none of those with carcinoma gave positive reactions to tuberculin plus cortisone. This is in contrast to the findings of Citron and Scadding (1957) in sarcoidosis, in which 50 per cent of the patients who were insensitive to tuberculin alone gave a positive reaction to tuberculin plus cortisone. This suggests that the mechanism of suppression of the tuberculin reaction in sarcoidosis is different from that in the reticuloses. The implications of these results are discussed. In a patient insensitive to tuberculin, the development of sensitivity when cortisone is added sug-

gests, but is not diagnostic of, sarcoidosis rather than one of the reticuloses (after authors' summary).—E. A. RILEY

Diagnostic Problems in Tuberculosis in the Aged (in Hungarian). H. JELLINEK*, P. TABÁK, and J. GIDÁLI. *Tuberkulozis*, April, 1960, 13: 104–106. (*U. of Budapest, Hungary)

Various pulmonary and extrapulmonary tuberculous processes were disclosed at autopsy in about 10 per cent of 1,000 persons who died when they were older than 60 years. Comparing the necropsy findings with the case histories, it was learned that 90 per cent of these lesions were not diagnosed while the patients were living. Tuberculosis was associated most frequently with malignant tumors, emphysema, and cor pulmonale, in that order.
—Z. VIRÁGH

UPPER RESPIRATORY TRACT DISEASE, VIRAL

Studies of the Epidemiology of Influenza as Demonstrated by Serum Pools. II. Evidence of Inapparent Infections and General Level of Population Resistance as Shown by the Antihemagglutination Reaction in Serum Pools Collected by Random Sampling. G. O. BROUN*, D. OLIGSCHLAEGER, M. LEGIER, and R. R. SCHMIDT. *Arch Intern Med*, October, 1960, 106: 496–512. (*St. Louis U. School of Med., Mo.)

As a result of this study of serum pools collected by random sampling between July, 1948, and the end of June, 1959, it appears that stimulation of antibody formation by a newly appearing virus or by anamnestic reaction to related viruses can be demonstrated in large pools of unselected serum almost as readily as in individual serum collected during an outbreak. This indicates a high degree of dissemination of the virus and a high percentage of inapparent infection, since a large majority of persons who contributed serum to the pools had no obvious antecedent influenza infection. Pre-epidemic levels of antihemagglutinin titer against the causative virus of an outbreak are lower than those of related viruses. The anamnestic rise in titer against related viruses during an outbreak often exceeds that of the causative virus. The rise in antibodies against the causative virus strain during an epidemic begins from a low level and is usually at least 4 times the pre-epidemic level. Absence or very low levels of antihemagglutinins against a given influenza virus strain for a period of years suggests absence of the virus from the human population. A virus may lose virulence and still stimulate antibody production, as indicated by a rise in antibodies against Asian viruses in early 1959 without the occurrence of an epidemic. A definite rise in antihemagglutinins against D-Sendai-1952-virus in 1949–1950, in 1955, and in 1957–1958, strongly suggests rather widespread exposure to this virus during these periods, although clinical outbreaks have not been recognized.
—W. J. STEININGER

Association of the Chimpanzee Coryza Agent with Acute Respiratory Disease in Children. M. BEEM*, F. H. WEIGHT, D. HAMRE, R. EGERER, and M. OEHME. *New Engl J Med*, September 15, 1960, 263: 523–530. (*Bobs Roberts Memorial Hosp. for Children, Chicago, Ill.)

An agent, herein termed Randall virus, antigenically similar to the chimpanzee coryza agent, was observed to be associated with a large portion of the acute respiratory illnesses seen at Bobs Roberts Memorial Hospital for Children in the winter of 1958–1959. The virus appeared to be responsible for the illnesses from which the patients suffered since it was isolated from the respiratory tracts of patients with acute respiratory illness, but not from those without respiratory illness. The virus could be demonstrated to be present in the respiratory tracts of some patients repeatedly during the course of the illness, it could not be demonstrated on convalescence, and it induced a significant increase in complement-fixing and neutralizing antibodies in the majority of patients from whom it was isolated. A variety of clinical patterns of respiratory illness was associated with isolation of this agent, but the most common were those of bronchiolitis and bronchopneumonia. The agent appeared to be present in the community between December, 1958, and April, 1959. This experience provides further indication that a virus antigenically similar to chimpanzee coryza agent produces infection and illness in human beings.
—M. J. SMALL

THORACIC SURGERY

Antecubital Strings—A New Physical Sign? H. G. EASTCOTT*. *Brit J Surg*, May, 1960, 67: 629–635. (*London, England)

After radical mastectomy, and in some conditions which affect the axillary lymph nodes, string-like bands may appear close beneath the elbow flexure. In 18 cases studied, the bands appeared to be due to a tightening and in some cases a thrombosis of the fine subcutaneous veins draining to the axilla. This condition closely resembles Mondor's disease of the breast. Mondor himself finally concluded that a stringy phlebitis was the cause. Lunn and Potter, reviewing the literature on Mondor's disease, conclude that the small ves-

sels concerned are probably always venous, and that the disease is self-limiting, although one may have to look for and treat the underlying cause. The author agrees with Kinmonth that the condition described in this paper may be regarded as Mondor's disease of the arm.—R. MacQuigg

Surgery in Broncho-Pulmonary Aspergillosis. J. R. Belcher* and N. S. Plummer. *Brit J Dis Chest*, October, 1960, 54: 335–341. (*London Chest Hosp., England)

The part played by *Aspergillus fumigatus* in bronchopulmonary disease in man is almost always that of a pure saprophyte, but it is sometimes capable of invading diseased tissues. The fungus has been found in an increasing number of cases presenting surgical problems, which may be divided into 3 groups: aspergillomas (simple and complex), aspergillar infection of suppurative lesions, and those cases in which the fungus was found in the mucous plugs characteristic of allergic aspergillosis. Complex aspergillomas develop in cavities with gross disease in the surrounding lung tissue. Hitherto, no distinction has been made between simple and complex aspergillomas, but the results of surgery in the 2 groups are sufficiently different to make the distinction important from a clinical point of view, quite apart from basic differences in pathology. Twenty-one cases of aspergillosis in which surgery had been carried out are reviewed, and the results are analyzed along with those in 22 cases from the literature. Applying the definitions outlined above, they have been classified as follows: simple aspergilloma, 6 cases; complex aspergilloma, 10 cases; secondary fungous infection, 2 cases; and allergic aspergillosis, 3 cases. The organism was isolated from the sputum before operation in 2 of the 6 patients with simple aspergillomas, in 6 of the 10 with complex aspergillomas, in 1 of the 2 in whom there was diffuse fungous invasion of suppurative lesions, but in none of the 3 patients with allergic aspergillosis. Surgery is relatively safe in patients with simple aspergillomas, but the incidence of postoperative complications in patients with complex aspergillomas combined with those who had aspergillar infection of suppurative lesions was very high (75 per cent). Two of the 9 patients who had complications died after operation. The indications for surgery in such cases are discussed. Surgery is neither necessary nor desirable for the localized lesions of allergic aspergillosis.—M. J. Small

Treatment of Oesophageal Atresia, A Modification in Technique. A. Sanderud*. *Acta Chir Scand*, August, 1960, 119: 339–342. (*Rikshospitalet, Oslo, Norway)

An anastomosis is performed over a catheter which is withdrawn when it is completed by a modification of the end-to-side technique, described by Sulamaa, Gripenberg, and Ahvenainen in 1952. It is thought that a wide lumen is provided with decreased danger of shrinkage at the anastomosis. This technique was used in 10 patients, with 1 postoperative death, and follow-up from 1 to 15 months in the others showed the anastomosis to be satisfactory in every case.—R. MacQuigg

Dilated Azygos Vein Simulating a Mediastinal Tumor: Report of a Case. M. H. Magbitang*, F. C. Hayford, and J. M. Blake. *New Engl J Med*, September 22, 1960, 263: 598–600. (*St. Peter's Hosp., Albany, New York)

Cases reported in the literature have included various causes for dilatation of the azygos vein simulating a mediastinal tumor, such as right-sided heart failure, obstruction of the superior and inferior venae cavae, and portal-vein hypertension. A case is presented of dilatation of the azygos vein to such a size as to strongly suggest a mediastinal tumor requiring surgery. At thoracotomy, the azygos vein was in its normal anatomic position but was approximately 10 times its normal size. The superior and inferior venae cavae were slightly smaller than normal. Several diagnostic aids in the differentiation of a dilated azygos vein from a mediastinal mass are enumerated. —M. J. Small

Five Year Survival After Surgery for Bronchogenic Carcinoma. D. Effler* and D. Barr. *Dis Chest*, October, 1960, 38: 417–422. (*The Cleveland Clinic, Ohio)

The data on 21 patients who survived 5 years or longer after surgery for bronchogenic carcinoma were examined in the hope of discovering factors influencing long-term survival. No feature consistently distinguished the survivors from the nonsurvivors, although lack of symptoms, peripheral location of the tumor, and the squamous-cell type of cancer were frequent findings in the survivors. Thirty-one per cent of the patients who underwent lobectomy lived 5 years, but only 16 per cent of those who underwent pneumonectomy lived that long.—E. A. Rouff

Extended Resection of Bronchogenic Carcinoma: A Reappraisal and Suggested Plan of Management. G. H. Lawrence*, J. H. Walker, and L. Pinkers. *New Engl J Med*, September 29, 1960, 263: 615–620. (*Virginia Mason Hosp., Seattle, Washington)

Surgical resection is the therapy of choice when bronchogenic carcinoma is grossly confined to the lung. Except in rare circumstances, lobectomy is the operation of choice in all lesions not requiring

intrapericardial pneumonectomy. Extended resection includes removal of not only the lung, but of the recognizable gross extension to the mediastinum, heart, thoracic parietes, or trachea, as well. Extended pneumonectomy is associated with an increased postoperative mortality and morbidity. Survival rates are not encouraging, and the operation should be reserved for the younger patient who can tolerate it best. Cobalt radiation therapy administered in selected cases not suited for standard surgical resection provides a hopeful means of palliation and possible cure.—M. J. SMALL

The Diagnostic Value of Pleural Biopsy in Bronchopulmonary Carcinoma. W. PAGEL* and S. GOLDFARB. *J Clin Path*, September, 1960, 13: 425–431. (*Clare Hall Hosp., Herts, England)

Of 26 patients with bronchopulmonary carcinoma, in 13 the diagnosis was either corroborated or arrived at by pleural biopsy. Taken as a whole, pleural biopsy is inferior to repeated examination of the pleural exudate for neoplastic cells. In individual observations, however, the former can be found positive at a time when neither the pleural fluid nor any other material provides a diagnostic clue.—E. DUNNER

Results of Radiotherapy in Inoperable Cases of Lung Cancer. J. KORHONEN*, K. AIRAKSINEN, P. KOSKINEN, and T. LEISTI. *Ann Med Intern Fenn*, 1960, 49: 131–135. (*Regional Hosp. of Central Finland, Jyväskylä)

Of 135 patients with pulmonary cancer seen at the Regional Hospital of Central Finland between 1954–1958, 9 were operated on elsewhere, 59 were given complete radiation therapy (skin dosage of 12,000 r), and 14 received palliative radiation therapy. The untreated cases totaled 53. Of the 59 patients given complete radiation therapy, 53 were dead by April, 1959. The mean survival time of the fatal cases after the initiation of therapy was 7.4 months. The mean follow-up period of the living patients was 9.8 months. None of the 14 patients receiving palliative radiation therapy were alive at the time of follow-up inquiry (mean survival, 2.8 months). Of the untreated patients, 2 were alive and 51 were dead (mean life of fatal cases, 3.9 months). It would seem that it is sometimes possible with radiation therapy to lengthen the lives of patients with inoperable cancer of the lung, although the results were poor with regard to complete recovery.—E. DUNNER

The Late Results of Heller's Operation for Cardiospasm. K. DOUGLAS* and F. NICHOLSON. *Brit J Surg*, November, 1959, 67: 250–253. (*Royal Infirmary, Manchester, England)

In 28 patients treated by Heller's operation, laparotomy was performed in 5 and thoracotomy in 23. When assessed 2 to 7 years after operation, there were 4 failures and 1 good result from laparotomy, and 4 failures, 10 good results, and 8 satisfactory results from thoracotomy. Recurrence was delayed for 3 years in 2 cases and for 6 years in 1 case, indicating that a long-term follow-up is necessary. It is important to see that all muscle fibers are divided to mucosa, and it is noted that the longer the myotomy on the esophageal side the better the result. If myotomy is done through a thoracotomy, then the hernia thus produced should be repaired by taking sutures in the hernial sac, passing them under the hiatus, and tying them on the under surface of the diaphragm through a counter incision in the diaphragm. This helps to prevent the reflux esophagitis which may complicate an otherwise excellent result.—R. MACQUIGG

The Prophylaxis of Pulmonary Embolism. N. M. GIBBS*. *Brit J Surg*, November, 1959, 67: 282–286. (*Royal Surrey County Hosp., Guildford, England)

Early ambulation has been only partially successful as a prophylactic measure. Early rising in the postoperative period, particularly in middle-aged patients, is an exhausting procedure. The active exercise obtained by a few faltering steps is paid for by hours of immobility while the patient recovers from the pain thus caused. Prolonged recumbency in arm chairs is even more dangerous than bed rest, since the risks of venous stasis are greater. Use of a "Soleus Ergometer" for exercising the calf muscles is recommended. Prolonged periods in the Fowler position should be avoided, particularly in obese patients, but the essential factor is the frequent changing of position. All investigators have found anticoagulant drugs effective in reducing thromboembolic complications, and especially when they are administered soon after admission to the hospital. The laboratory control of such therapy precludes its routine use at present, but some patients present special problems, such as obesity, congestive cardiac failure, injuries to the lower limb, or pregnancy, whereby adequate active mobility is difficult to achieve.—R. MACQUIGG

Herniation in Congenital Diaphragmatic Defects. J. A. W. BINGHAM*. *Brit J Surg*, July, 1959, 67: 1–15. (*Belfast, Ireland)

Posterolateral defects, the most common, lie at the periphery of the diaphragm in the region of its attachment to the tenth and eleventh ribs. Only rarely is there a pleuroperitoneal sac. In the majority of cases, urgent operative treatment is required as soon as possible after birth. Diagnoses can be made from chest roentgenograms taken of

newborns with dyspnea by the presence of gut in the chest. Thoracoabdominal incision in the line of the tenth interspace is recommended as the best approach. The midgut is usually not attached to the posterior abdominal wall and may be malrotated, and in these cases it is advisable to carry out Ladd's procedure (division of the peritoneal fold passing across the duodenum in order to prevent later duodenal obstruction or midgut volvulus). The use of excessive pressure to inflate the lungs should be avoided as it may cause a tension pneumothorax. In all of the cases described, one-stage closure proved possible. It is important to withhold oral feeding until the tense abdomen becomes soft.—R. MacQuigg

Hiatus Hernia: A Cause of Gastro-Intestinal Hemorrhage. P. Marchand*. *Brit J Surg*, March, 1960, 67: 515–525. (*Johannesburg General Hosp., South Africa)

This review confirms that esophageal erosion due to reflux does not cause massive bleeding. Thirty-seven cases of hemorrhage in a total series of 138 cases of hiatal hernia are described. Seven patients were admitted with unexplained anemia, and 19 had bled massively. Most of the patients with massive hemorrhages or severe anemia had large rolling hernias. All of the massive hemorrhages and severe anemia not caused by duodenal ulcers were due to ulceration of the mucosa within the herniated stomach. A "blind" partial gastrectomy should never be performed for hemorrhage unless the hiatus has been adequately explored. —R. MacQuigg

Leiomyoma of the Lung (in Japanese). H. Sawazaki*, G. Tajima, A. Onoki, and T. Ishiko. *Jap J Thor Surg*, November, 1960, 13: 934–939. (*Kanto Teishin Hosp., Tokyo, Japan)

A case is presented of primary leiomyoma of the lung, which is a rare tumor. This is the twelfth case of primary benign leiomyoma of the lung reported together with photomicrographic illustrations. It is the first case diagnosed by bronchoscopic biopsy in the literature; the other 11 cases were not definitely diagnosed before operation or autopsy. The patient was a 47-year-old housewife who came to the clinic complaining of productive cough and fever in February, 1960. A chest roentgenogram showed the left lung to be totally opaque. Bronchography revealed occlusion of the left main bronchus just below the bifurcation. On bronchoscopic examination, the left main bronchus was blocked by a grayish-white tumor with a flat surface. A biopsy specimen taken from the tumor showed interlacing whirled bundles of elongated spindle-shaped cells. With the use of van Gieson and Azan-Mallory staining techniques, this case was diagnosed finally as a leiomyoma of the lung. Surgery is scheduled.—I. Tateno

Primary Leiomyosarcoma of the Lung (in Hungarian). G. Mészáros* and J. Simárszky. *Tuberkulozis*, May, 1960, 5: 144–148. (*Tbc. Klinika, Debrecen, Hungary)

The case history is presented of a 45-year-old man in whom an egg-sized and egg-shaped shadow was discovered by mass survey in the left upper hilar area. The patient was asymptomatic. Exploratory thoracotomy and extemporaneous pneumonectomy were done, and the histopathologic diagnosis of the resected tumor was leiomyoma sarcomatosum. There have been 25 cases of pulmonary leiomyosarcoma reported in the literature. —Z. Virágh

A New Method of Controlling Paradoxical Respiration in Single Stage Thoracoplasty. B. Burch* and L. Shefts. *Dis Chest*, September, 1960, 38: 261–263. (*Patton State Hosp., California)

There is still a place for thoracoplasty in the management of the poor-risk patient who is not acceptable for resectional therapy. A single-stage thoracoplasty is accomplished and paradoxic respiration is controlled by the simple maneuver of placing sutures about the individual intercostal muscles which are anchored posteriorly in the sacrospinalis muscle and anteriorly in the sternal origins of the pectoralis major. Three stages may thereby be done in a single operation. Of 23 advanced cases of tuberculosis so treated, 1 died of a massive pulmonary hemorrhage 6 days postoperatively, and 1 reactivated a month after surgery; the remaining 21 cases have remained bacteriologically negative, the longest follow-up being 4 years. An additional 12 nontuberculous patients were operated on in this manner without complications.—E. A. Rouff

Successful Surgery in Giant Pulmonary Tuberculoma Simulating a Posterior Mediastinal Tumor (in Japanese). Y. Kurihara* and K. Hirano. *Clin Resp Organs*, September, 1960, 15: 641–645. (*Hiroshima Municipal Social Insurance Hosp., Japan)

The authors report successful resection of a localized solitary giant tumor in the left lung of a 37-year-old male patient. The preoperative diagnosis was posterior mediastinal tumor. The giant, fist-sized tumor was in the lower lobe; it was filled with a chocolate-like mucoid substance and was not considered to be a tuberculous lesion macroscopically. However, the histologic diagnosis was tuberculosis with giant cells, cell infiltration, caseation, and cavity formation. The differential diag-

nosis between malignant tumors and tuberculoma is discussed, and early surgical intervention is recommended when the diagnosis is uncertain because many localized solitary pulmonary shadows encountered were proved to be due to malignant lesions.—I. Tateno

Results of Pneumonectomy and an Analysis of 220 Cases in Japan (in Japanese). T. Wada* and S. Komatsu. *Clin Resp Organs*, September, 1960, 15: 605–610. (*Sapporo Med. College, Japan)

The authors analyzed pneumonectomies performed by themselves and by other surgeons in Japan. This operation has been used increasingly over the past 10 years. Eighty per cent of the pneumonectomies were performed for pulmonary tuberculosis, and 90 per cent of the patients who survived the operation were completely cured. The incidence of the development of complications was between 20 and 30 per cent, but 90 per cent of these patients were clinically cured 1 year after operation. According to the authors' experience, the presence of increased pulmonary artery pressure before operation was not a contraindication to the operation. There was no close correlation between preoperative vital capacity and death rate. The presence of drug-resistant tubercle bacilli in the sputum was often associated with postoperative complications, but most such patients were cured 1½ to 2 years after operation. The presence of drug-resistant tubercle bacilli was, therefore, not a contraindication to operation when final cure of the disease was the aim. The mortality rate was about 10 per cent and death usually occurred soon after operation. The number of pneumonectomies performed for lung cancer, pulmonary infection, and bronchiectasis has been limited in Japan, making a critical analysis impossible.
—I. Tateno

Pneumonectomy in Children and Adolescents (in German). H. Brügger*, H. Duesel, and P. König. *Beitr Klin Tuberk*, April, 1960, 122: 77–93. (*Kinderheilstätte Wangen [Allgäu], Germany)

This is a report on 107 pulmonary resections performed in children aged 7 to 19 years. There were 7 pneumonectomies; in 2 of these patients only lobectomy was indicated preoperatively. In 6 children, the chest cavity was refilled with air after pneumonectomy until shrinking of the chest, shift of the mediastinum, elevation of the diaphragm, and narrowing of the intercostal spaces obliterated the empty hemithorax. Thoracoplasty is not recommended—and none was attempted—for the purpose of decreasing the dead space in the chest cavity after pneumonectomy, as extensive thorax deformity is likely to result. Filling the hemithorax with plombage material should also be avoided because of the danger of infection.
—Z. Virágh

Manpower Salvage with Surgery in Pulmonary Tuberculosis. D. E. Thomas* and E. M. Aronstam. *JAMA*, September 3, 1960, 174: 23–25. (*Valley Forge Army Hosp., Phoenixville, Penn.)

A 5-year follow-up study of selected active military personnel revealed that excisonal surgery for pulmonary tuberculosis resulted in a mortality of less than 1 per cent, a cure rate of 97 per cent, and a work rehabilitation of 98 per cent among those whose disease remained inactive. These figures are based on an experience with almost 400 patients operated on between 1953 and 1957.—H. Abeles

Surgical Indications in Pulmonary Tuberculosis (in Roumanian). N. Bumbăcescu* and C. Ionescu. *Ftiziologia*, March–April, 1960, 2: 139–152. (*Clinica de Ftiziologie, Iaşi, Roumania)

The records of 10 patients, including progress reports and roentgenograms, were sent to 40 phthisiologists and 17 thoracic surgeons for their opinions as to the treatment to be recommended. Resectional surgery was suggested most often, and was followed by the various forms of surgical collapse. In 1 patient, pneumothorax was indicated by 3 physicians, but none of the surgeons concurred. Both the physicians and surgeons agreed on preoperative treatment with antimicrobials for 6 to 7 months.—Z. Virágh

Electrolyte Concentration in the Pleural Fluid and Electrolyte Balance after Pulmonary Resection for Tuberculosis (in Japanese). Y. Yamada*. *Kekkaku*, September, 1960, 35: 641–648. (*Keio U. School of Med., Tokyo, Japan)

Electrolyte balance studies on both the serum and pleural fluid of uncomplicated surgical cases were conducted, and the following conclusions were reached. Sodium concentration in the pleural fluid ranged from 131.8 to 144.8 mEq/l and was always lower than that in the serum. Chloride concentration ranged from 95.5 to 115.8 mEq/l and was always higher than that in the serum. Potassium concentration ranged from 3.2 to 10.3 mEq/l and there was no correlation between its concentration in the pleural fluid and in the serum. The blood volume in the pleural fluid as determined by hematocrit and hemoglobulin determinations fell rapidly after the second postoperative day, and the mixing ratio of the exudate increased remarkably. The patients who received 300 to 500 ml. more blood than they lost during and

after operation did not show any significant changes in serum electrolytes. Sodium retention lasted for about 3 or 4 days after operation, in general, but the degree and duration of sodium retention was more prominent when the pleural exudate was higher (high mixing ratio of pleural exudate and blood). Increased potassium excretion lasted until the second or third postoperative day. Recovery to the preoperative levels was usually seen by the seventh to tenth day after operation. Eosinophilic cells fell sharply to 10 or less per cu. mm. after operation. The correlation between the eosinophilic response and the changes in urinary sodium excretion was noted.—I. Tateno

Anterior Spine Fusion for the Treatment of Tuberculosis of the Spine. A. R. Hodgson* and F. E. Stock. *J Bone Joint Surg (Amer)*, No. 2, 1960, 42-A: 295–310. (*U. of Hong Kong)

The operative findings and results of treatment of 100 patients with tuberculosis of the spine seen at the University of Hong Kong are reported. The usual methods of abscess drainage, removal of bone sequestrae, antimicrobials, and bone fusion were employed. The superior results from anterior spine fusion compared with conservative methods or posterior fusion are stressed. It is noted that the anterior approach gives wide access to the disease, which is always more extensive than the roentgenogram would lead one to suppose. Likewise, the authors state that anterior fusion by their method has reduced the period of hospitalization from years to months. In 11 patients previously treated by posterior fusion, activity of the disease in the spine was demonstrable despite roentgenographic evidence of solid fusion. In the group of 100 patients, there were 4 deaths, 2 non-unions, and 94 successful fusions; 1 patient required 2 operations. —B. Rocoff

Thoracic Surgery in Mentally Ill Patients. O. Bettag*, J. Lloyd, D. Silva, and L. Krasner. *Dis Chest*, October, 1960, 38: 362–369. (*Illinois Dept. of Public Welfare, Chicago)

Over a 10-year period, 388 surgical procedures were performed on 343 mentally ill or retarded patients, with an over-all postoperative mortality (within 90 days) of 6.7 per cent. The over-all percentage of postoperative mortality for tuberculosis patients was 5.6 per cent (representing a postresection mortality of 7.9 per cent). Good results were obtained in 82 per cent of the cases of tuberculosis (295 procedures on 251 patients). The postoperative management of these patients is especially important when dealing with the more severe mental defectives and the passive chronic psychotic patients.—E. A. Rouff

Prevention and Treatment of Respiratory Complications in Surgery. B. B. Roe*. *New Engl J Med*, September 15, 1960, 263: 547–550. (*U. of California School of Med., San Francisco)

Major pulmonary complications continue to be a significant hazard to surgical patients, and occult complications are likely to be more common than is generally appreciated. An intensive program of prevention and treatment is recommended. Traditional aspects of surgical management that are prejudicial to respiratory function and predispose to complications should be discarded. Wider application of airway moisture, expectorants, tracheal aspiration, bronchoscopy, and tracheostomy is encouraged for prophylaxis as well as treatment (Author's summary).—M. J. Small

Surgical Significance of the Thymus. D. P. Boys* and F. P. Gerard. *New Engl J Med*, September 1, 1960, 263: 431–436. (*Lahey Clinic, Boston, Mass.)

Twenty-four thymectomies were performed from 1928–1958. Seven were in patients with myasthenia gravis who had failed to respond to medical measures. The remaining 17 were for tumors of the anterior mediastinum, 4 of which were associated with myasthenia gravis. It is concluded that thymectomy appears to be of definite value in the treatment of myasthenia gravis in the properly selected patient. The patient most likely to have a good result is the woman less than 35 years of age in whom the disease has been present for less than 5 years. An associated thymoma is a grave prognostic sign. Despite the poor prognosis, thymic tumors should be excised if possible, for life expectancy without removal is short. Tumors of the anterior mediastinum should be explored whether they are considered benign or malignant. Excision of an encapsulated tumor offers the best chance for survival. Thymoma must be considered a highly malignant lesion, as is evidenced by the fact that half the patients with thymomas who were operated on were dead within 6 months. —M. J. Small

The Sheep as an Experimental Animal in Surgical Science. J. Borrie* and R. M. Mitchell. *Brit J Surg*, January, 1960, 67: 435–445. (*Otago Med. School, Dunedin, New Zealand)

Sheep were used in more than 800 survival operations in the Department of Surgery at the University of Otago, New Zealand. Dogs were not used, not only because of the noise they would cause in the city area and the heavy maintenance cost for long-term experiments, but also because of limited holding space in the Medical School precincts. Animals heavier than 80 to 100 pounds were avoided, especially for abdominal work, be-

cause of excessive intra-abdominal fat. Preoperative starvation was increased to 48 hours, due to a tendency to operative and postoperative regurgitation from the enormous stomach. A Palmer anesthetic pump was used for thoracic operations. A check list is given of danger points in the anesthetic technique. Atropine and pentobarbitone sodium are injected into either external jugular vein. The sheep may be very easily cooled and rewarmed for hypothermic experiment. If prolonged intercostal water-seal drainage with suction is necessary, the animal is easily supported on a Cyclone ewe holder, and can remain there up to 24 hours if necessary. At the conclusion of the experiments the wool is marketed, but not the meat, as wages for butchering exceed receipts from its sale. Suggestions are given for urine collection.
—R. MacQuigg

ROENTGENOGRAPHY

Selective Terminal Angiopneumography (in Spanish). S. Bravo Olalla*, L. Castro García, and E. Pérez Rula. *Enferm Toraz*, No. 34, 1960, 9: 145–158. (*Dispensario de Tórax, San Sebastian, Spain)

Preoperative function studies of the right heart and lesser circulation are indispensable in pulmonary surgery. Selective terminal angiopneumography would help to forestall certain postoperative complications by revealing the circulatory condition of those parts of the lung which are expected to function properly after the operation. The technique of the procedure is described and illustrated with angiopneumograms of 4 patients.—Z. Virágh

Modification of Bronchographic Technique (in Spanish). J. Abello*. *Rev Esp Tuberc*, July, 1960, 29: 303–317. (*Sanatorio Helios, Guadarrama, Spain)

After introducing a Metrax catheter into one of the main bronchi, a fine plastic tube was passed, with the help of a metal wire, to the desired lobar or segmental bronchus or to one of its principal ramifications. Once the plastic tube was in place, non-oily contrast material was injected into it without pressure. Using this method, good roentgenograms can be obtained even of the bronchioli. The first experiments were carried out in dogs, and were later performed successfully in humans.
—Z. Virágh

Aimed Bronchography. O. Kassay*, M. Erdelya, and R. Schuster. *Dis Chest*, October, 1960, 38: 382–390. (*Lankenau Hosp., Philadelphia, Penn.)

Specially constructed tubes of varying degrees of pliability and angulation have been made for guided bronchoscopic insertion under fluoroscopic control. They have proved to be of distinct value when suspicious lung areas have not been filled by conventional bronchographic means.—E. A. Rouff

Intercostal Lung Bulging, an Early Roentgen Sign of Emphysema in Children. S. Schorr* and D. Ayalon. *Radiology*, October, 1960, 75: 544–551. (*Municipal Hosp. "Hadassah," Tel-Aviv, Israel)

In the normal roentgenogram, the line separating the radiolucent lung from the intercostal soft tissue is straight or at times slightly concave. Convexity or outward bulging of this borderline appears to indicate overexpansion of the lung, the so-called "air trapping" or acute emphysema of children. This sign was noted in 27 infants and young children: 22 had clinical and other roentgenographic evidence of acute inflammatory processes in the respiratory tract, 5 had no other roentgenographic abnormality. In 18 of these cases there were associated roentgenographic signs of pulmonary emphysema. In 6, the lateral chest film revealed parasternal intercostal bulging, not always accompanied by bulging of the lateral intercostal spaces. The diagnosis of acute pulmonary emphysema was verified in 5 autopsied cases. Intercostal bulging is an expression of emphysema of the peripheral layers of the parenchyma due to temporary obstruction of the small bronchioles by mucous plugs or inflammatory edema of the bronchiolar mucosa. The emphysema is reversible. This roentgenographic sign may denote an early stage of lung overexpansion and may be an indication of subclinical lung inflammation.
—W. J. Steininger

Combined Fluoroscopy and Endoscopy of the Bronchi and Esophagus (in German). H. L. Wullstein*. *Deutsch Med Wschr*, July 22, 1960, 85: 1329–1330. (*Luitpoldkrankenhaus, Würzburg, Germany)

This is a description of a fully mobile table and roentgen apparatus with image amplifier and television screen which makes possible endoscopic examination of the esophagus and bronchi simultaneously with the visualization of the same area on the television screen as fluoroscopy image. This facilitates the performance of any operations in the bronchi or the esophagus.—J. Haapanen

The Functional Effect of Pulmonary Irradiation. M. Sutton*. *Brit Med J*, September 17, 1960, No. 5202: 838–841. (*Hammersmith Hosp., London, England)

Serial lung function studies were performed on patients who were having their lungs irradiated for carcinoma of the bronchus or carcinoma of the

breast. Lung function tends to be depressed toward the end of the course of radiation therapy at 240 kV. Such a depression is not seen with supervoltage radiation therapy. This is probably due to the difference in radiation reaction. Moderate functional disability results from direct pulmonary irradiation by supervoltage roentgen rays as measured at the end of 1 year. No real functional disability results from radiation therapy for carcinoma of the breast when modern radiation therapy techniques are employed (after author's summary).—E. A. Riley

Anomalous Drainage of Pulmonary Veins into the Inferior Vena Cava. Importance of Planigraphic Study. W. Koch* and A. Silva. *Radiology*, October, 1960, 75: 592–594. (*Carlos Van Buren Hosp., Valparaiso, Chile, S.A.)

Two cases are presented of anomalous venous drainage of the right lung into the inferior vena cava. Both had associated congenital anomalies. In one there was an anomalous distribution of the bronchi to the right lung and small anomalous vessels from the systemic circulation of the mediastinum to the right lung. The other had an interatrial septal defect. Planigraphic studies were of value in establishing the correct diagnosis. —W. J. Steininger

Reflections of Disease in the Pulmonary Medulla. R. P. Barden*. *Radiology*, September, 1960, 75: 454–455. (*Chestnut Hill Hosp., Philadelphia, Penn.)

Characteristic but nonspecific roentgenographic shadows in the lungs may occur in certain "hypersensitivity states" such as periarteritis nodosa, and in toxic states such as uremia. These shadows were thought to be due to a reversible edema which occupied the central portion of each lung in a peculiar, symmetric manner. Recent studies show that the lung is divided into two parts: a peripheral zone, or cortex, and a central medullary zone which is rich in capillaries. All conditions producing increased capillary permeability will result in selective leakage of fluid in the medullary zone. In conditions such as passive congestion, in which edema fluid collects in peripheral portions of the lungs and in the pleural cavities, one may assume that the mechanism of leakage is different and that vessels larger than capillaries are primarily involved.—W. J. Steininger

CHEMOTHERAPY

The Nineteenth Veterans Administration—Armed Forces Conference on the Chemotherapy of Tuberculosis. E. Rothstein*. *New Engl J Med*, September 22, 1960, 263: 588–591. (*V. A. Hosp., Brockton, Mass.)

The Nineteenth Conference, like its predecessors, devoted the major portion of its time to the chemotherapy of tuberculosis. There was an increasing interest in the broader field of respiratory disease, however, reflecting the fact that, with the shortened hospital stay and decreased bed occupancy by tuberculous veterans, the staffs of the Veterans Administration hospitals are caring for more patients with chronic nontuberculous illnesses of the respiratory tract. The major findings of the conference are summarized as follows: Randomized studies to date have not shown any advantage in high-dose isoniazid therapy, although toxic reactions were increased. Corticosteroids may be used as part of the treatment of pulmonary tuberculosis. They are occasionally of benefit and usually not harmful, but are of doubtful ultimate value. In the presence of effective drug therapy, results are very good despite the absence of rest treatment. In patients with resistant tubercle bacilli, occasional benefit, chiefly bacteriologic, may be expected from thioamide (ethionamide, TH1314) or from cycloserine in large doses. Amphotericin B is of value in the treatment of pulmonary histoplasmosis. Large-scale cooperative studies are being utilized in the evaluation of diagnostic, therapeutic, and prognostic programs dealing with many respiratory illnesses—chronic bronchitis, histoplasmosis, emphysema, and bronchogenic carcinoma.—M. J. Small

The Influence of Antimicrobials on Bacilloscopy (in Spanish). J. Padilla Manzuco*. *Enferm Torax*, No. 34, 1960, 9: 175–188. (*Sanatorio de La Fuenfria, Madrid, Spain)

Chemotherapy is liable to delay the development of colonies of tubercle bacilli in culture media. Therefore, it is recommended that the cultures should be kept for 2 to 3 months.—Z. Virágh

Acceptability of Ethionamide (1314TH) in Nigerian Patients. J. R. Lauckner*. *Tubercle*, October, 1960, 41: 367–369. (*University College, Ibadan, Nigeria)

Ethionamide in a daily dosage of 15 to 20 mg. per kg. per day appears to be better tolerated by Nigerian than by European patients. No serious toxic effects have been observed in some 30 patients treated thus far. The patients did not often complain of anorexia, nausea, vomiting, epigastric pain, or diarrhea, symptoms which have been reported to cause considerable difficulty in European patients. At various stages between the third and eleventh weeks of treatment, 7 patients complained of either sore mouth or sore tongue. In its milder form there was no visible abnormality.

When more severe, there was redness of the buccal mucosa with some loss of normal furring of the tongue and, sometimes, angular stomatitis or cheilosis. On the basis of clinical resemblance to deficiency of B vitamins, yeast was prescribed while continuing ethionamide. In 6 cases there was considerable improvement or complete clearing of signs and symptoms. One-half of the patients treated showed roentgenographic deterioration, 5 showed little change, and 1 showed considerable improvement. Loss of weight does not appear to be clearly related to clinical deterioration. Furthermore, loss of weight tended to be continuous during the period of administration of the drug and did not follow the fall and rise of sputum positivity. Five of the patients treated gained considerable weight during the succeeding 3 months, and none continued to lose weight after treatment with ethionamide was stopped. These observations suggest that loss of weight or failure to gain was directly related to the ethionamide, irrespective of clinical improvement or deterioration. In many cases, weekly examination of direct sputum smears prepared according to a standard technique showed a pronounced fall-and-rise phenomenon, suggesting that ethionamide administered alone has a definite tuberculostatic effect and that resistance develops rapidly. Ethionamide has several properties which recommend it as a potentially useful drug for the treatment of tuberculosis in the tropics. Its use in this connection should be further explored.—M. J. SMALL

The Susceptibility to Hydrogen Peroxide of Indian and British Isoniazid-Sensitive and Isoniazid-Resistant Tubercle Bacilli. T. V. SUBBAIAH*, D. A. MITCHISON, and J. B. SELKON. *Tubercle*, October, 1960, 41: 323–333. (*Tuberculosis Chemotherapy Center, Madras, India)

A standard, bactericidal, hydrogen peroxide susceptibility test was developed in which a viable count was done on strains that had been exposed to 0.02 per cent hydrogen peroxide for 90 minutes at 37°C. This test has been found to estimate accurately the proportion of catalase-positive, isoniazid-susceptible organisms in a mixture with catalase-negative, isoniazid-resistant organisms. Among strains from British patients, 8 with full susceptibility to isoniazid were uniformly resistant to peroxide, and 7, which were isoniazid-resistant but still retained some catalase activity, were more susceptible to peroxide, but contained at least 0.11 per cent peroxide-resistant organisms. In contrast, among strains from Indian patients, 4 of the 7 isoniazid-susceptible, catalase-positive strains were more susceptible to peroxide than were any of the British strains, and 3 of the 8 isoniazid-resistant, catalase-positive strains contained 0.018 per cent or fewer peroxide-resistant organisms. All 9 catalase-negative, isoniazid-resistant strains from both British and Indian patients were completely susceptible to peroxide. An association was found between low catalase activity, high susceptibility to peroxide, and a high degree of resistance to isoniazid. This association was more clear cut in the British than in the Indian strains. The relationship between peroxide susceptibility and animal virulence in the Indian strains is being investigated. This is of particular interest, since it has been shown that isoniazid-susceptible Indian strains of tubercle bacilli, of similar origin to those reported here, had a wide range of virulence in the guinea pig and were frequently less virulent than British strains.—M. J. SMALL

Concerning Jaundice in Iproniazid Treatment with Consideration of a Case of So-Called Allergic Cholangitis (Drug Icterus) (in German). H. THALER*. *Wien Klin Wschr*, August 19, 1960, 72: 588–591. (*I. Medizinische Universitaetsklinik, Vienna, Austria)

Iproniazid has a damaging effect on the liver parenchyma; this effect may aggravate or activate a virus hepatitis. The case history is presented of an 86-year-old man who had severe angina pectoris and was given iproniazid treatment for 10 days. Thirteen days after the onset of treatment the patient developed icterus. A liver biopsy revealed the presence of acute cholangiolitis. The icterus subsided after a few days. The patient died 3 weeks later from cardiac failure. At autopsy, the liver was found to be normal. It is believed that in this case iproniazid caused an allergic cholangiolitis.—G. C. LEINER

Kanamycin and Streptomycin in the Treatment of Pulmonary Tuberculosis (in French). H. BONSTEIN* and G. FAVEZ. *Schweiz Z Tuberk*, No 3, 1960, 17: 179–185. (*Clinique Sylvana, Epalinges, Vaud, Switzerland)

The therapeutic results obtained from the treatment of 20 patients with kanamycin and PAS infusions compared favorably with those in 20 other patients treated with streptomycin and isoniazid. Kanamycin appeared to be more toxic.
—Z. VIRÁGH

Hypersensitivity to Para-Aminosalicylic Acid. T. K. KRZYSKI* and R. C. YOUNG. *Canad Med Ass J*, September 3, 1960, 83: 527–530. (*Point Edward Hosp., Sidney, N.S.)

Of 894 patients who received PAS therapy for tuberculosis during the period from January 1, 1954, to December 31, 1958, 33 developed hypersensitivity and clinically recognizable reactions to this antimicrobial agent, an incidence of 3.6 per

cent. The type of reactions observed varied, acute fever and skin eruption being the most common. Several patients had severe gastrointestinal symptoms and 2 developed hepatotoxicity. The average length of time for hypersensitivity to become evident was 40 days, with an average ingestion of 385 gm. of PAS. The shortest time interval from initiation of therapy to reaction was 24 hours. Hepatotoxicity is probably the most serious manifestation of PAS hypersensitivity. It usually occurs after a large daily dosage and continuance of drug administration after the first manifestations of reactions have appeared. The importance of discontinuing PAS at the earliest sign of possible allergic reaction cannot be emphasized too strongly (after authors' summary).—E. A. Riley

Prednisolone in the Treatment of Acute Extensive Pulmonary Tuberculosis in West Africans. W. J. Bell*, P. P. Brown, and D. W. Horn. *Tubercle*, October, 1960, 41: 341–351. (*Tuberculosis Research Unit, Accra, Ghana)

A controlled trial was undertaken to determine whether prednisolone, added to standard chemotherapy, hastened clinical and roentgenographic improvement and cleared the sputum more rapidly of tubercle bacilli in acute extensive pulmonary tuberculosis in West Africans who had only a short period of hospital treatment. Forty-nine patients were treated with prednisolone combined with chemotherapy, and 51 with chemotherapy alone. The dose of prednisolone was 5 mg. 4 times daily for 8 weeks, tapering off by the tenth week. Chemotherapy consisted of daily streptomycin, PAS, and isoniazid (300 mg.) for 12 weeks and, thereafter, isoniazid–PAS. Clinical improvement was more rapid in the prednisolone-treated group, particularly in those patients severely ill or in poor condition during the first month of treatment. There were no significant differences with respect to weight change, roentgenographic clearing, or cavity closure. Clearance of bacilli from the sputum was not hastened, and an initial rise in the bacterial count was noted in the prednisolone-treated cases. A well-marked rise in the circulating leukocytes (including a rise in eosinophils) was observed in the prednisolone cases. Prednisolone may have a place in effecting acceleration of improvement in the general condition of those who are severely ill, but from the evidence of this investigation it would seem that, in the dosage given and in the type of disease investigated, it has no other place in the routine early treatment of pulmonary tuberculosis in the West African.—M. J. Small

Hyperuricemia Due to Pyrazinamide. J. Schneeweiss* and G. W. Poole. *Brit Med J*, September 17, 1960, No. 5202: 830–832. (*Hammersmith Hosp., London, England)

Pyrazinamide causes a rise in serum uric acid and a reduction in urinary excretion of uric acid within about 48 hours. Within a similar period of the suspension of this drug, these figures revert to the normal range. This effect was reduced by the concurrent administration of probenecid in standard dosage. In this series neither PAS nor isoniazid was found to have any effect on the level of uric acid in the serum. Pyrazinamide appears to act by interfering with the kidneys' ability to concentrate and excrete uric acid in the normal manner, presumably by increasing the tubular reabsorption of uric acid. It has been suggested that, after prolonged pyrazinamide administration, decreased urate excretion persists; apparently, this also involves decreased production (Gleason *et al.*, 1956). This could be a physiologic response to elevated serum uric acid levels, but may represent a more fundamental disturbance in urate metabolism. Special care should be exercised in the use of pyrazinamide in patients with a past or family history of gout, as the development of hyperuricemia may lead to the precipitation of clinical attacks of gout (after authors' summary).—E. A. Riley

A Maintenance Trial of Combined Steroid and Antibiotic Treatment in a Series of Asthmatics with Chronic Bronchitis. A. G. Ogilvie* and D. J. Newell. *Brit J Dis Chest*, October, 1960, 54: 308–320. (*Royal Victoria Infirmary, Newcastle-upon-Tyne, England)

Sixty men less than 65 years old who had persistent asthma and chronic bronchitis were treated with a combined regimen of antimicrobials—novobiocin, oxytetracycline (Terramycin®), and an unmarked tablet which was either methylprednisolone (4 mg.) or a dummy—for a trial period of 4 winter months. The criteria of selection for the trial were fitness for the job on entry, and a report by the patient of a reasonable amount of loss of work the preceding 2 winters during the months corresponding to those of the trial period. The sole criterion of improvement recognized was a comparison of the amount of sick absence during the trial period as compared with the 2 preceding winters. Discrepancies between the records kept by the patients and those supplied by the Ministry of Pensions and National Insurance regarding absence from work were fairly numerous and applied to 20 per cent of the cases. The result of the trial was affected by this discrepancy, but even if the most favorable estimate had been accepted, the economic gain would have been relatively slight. At present costs, the use of methyl-prednisolone would make for no economic gain, according to the Ministry's results. An anal-

ysis of the effect of the antimicrobial indicated no statistically significant change.—M. J. Small

Bacterial Resistance to Streptomycin, PAS and Isoniazid in Untreated Patients with Symptomatic Pulmonary Tuberculosis in Ashanti. W. J. Bell* and P. P. Brown. *Tubercle*, August, 1960, 41: 247–252. (*Tuberculosis Research Unit, Ghana, West Africa)

This paper, as a preliminary to resistance studies of a broader nature being undertaken throughout Ghana, gives the results of sensitivity testing of previously untreated cases of symptomatic pulmonary tuberculosis that were treated at the Kumasi Central Hospital, Ashanti, Ghana. Of 343 positive cultures tested, 53 (15.4 per cent) showed some degree of resistance to either streptomycin, PAS, or isoniazid. Resistance to a single drug was most common; resistance to 2 or 3 drugs was found in 32 per cent of all resistant cultures. Isoniazid resistance was most common, but was almost equalled by streptomycin resistance. A 10 per cent rise was demonstrated in less than 1 year. Many of the resistant patients treated were friends or relatives of patients already receiving treatment at home and living with them in close, unsanitary conditions. The disturbing facts are the high rates of existing resistance and increasing resistance in Africa; for, with present facilities, there is no alternative in the treatment of pulmonary tuberculosis other than to apply relatively uncontrolled outpatient chemotherapy. In East Africa, between the years 1953 and 1957, there was an increase from 0 to 12.9 per cent in PAS resistance, and a similar increase of 16 per cent in isoniazid resistance. Adding to the individual problem and to the problem of increasing resistance in general is the fact that, while the present practice of using isoniazid/PAS combinations as "standard" therapy is continued, an 11 per cent chance exists that inadequate therapy will be given.—M. J. Small

Experimental Studies on the Antituberculous Activity of Long-acting Sulfanilamide Derivatives (in Japanese). H. Sugiura*. *Kekkaku*. October, 1960, 35: 688–695. (*Institute for Infectious Diseases, Tokyo U., Japan)

The susceptibility of tubercle bacilli grown in Youmans' liquid medium to various long-acting sulfanilamide drugs was tested. The minimal growth-inhibiting concentrations of these compounds were between 25 and 50γ per ml. The inhibitory effects of sulfadimethoxine, sulfisomezole, and sulfamethizole were greater than those of sulfisoxazole, sulfaphenazole, or sulfamethoxypyridazine. Tubercle bacilli resistant to streptomycin, isoniazid, and PAS were equally susceptible to the sulfanilamides. They had synergistic inhibitory action with isoniazid for the growth of tubercle bacilli and prevented the development of isoniazid resistance. The blood specimens taken after oral administration of the sulfanilamides had an inhibitory effect on tubercle bacilli when tested by the slide-cell culture method; the effect was especially remarkable when sulfamethoxypyridazine, sulfadimethoxine, or sulfaphenazole were given orally. Blood specimens taken after oral administration of sulfanilamide and isoniazid were more inhibitory than those obtained after isoniazid administration.—I. Tateno

Clinical Studies on Thiamine Ethyl Disulfide (in Japanese). K. Yamada*, H. Sawaki, and O. Yasaki. *Jap J Chest Dis*, September, 1960, 19: 653–658. (*Nagoya U. School of Med., Japan)

Thiamine ethyl disulfide (TED) was put on clinical trial in pulmonary tuberculosis because of its promising therapeutic effects in *in vitro* and *in vivo* experiments in guinea pigs. Seventeen patients were given TED, which was well tolerated in a dosage of 2.0 to 3.0 gm. daily for 6 months. No toxic effects on the bone marrow and liver, or the digestive, cardiovascular, urogenital, or nervous systems were found. Nine patients were given TED alone, and 8 were given TED in combination with other drugs. Most patients showed roentgenographic improvement, but the illness worsened in 1 patient. Of the 9 patients who had sputum positive for tubercle bacilli at the onset of treatment, the bacilli became negative in the sputum of 4 patients and decreased in number in 2. There was a decrease in cough, sputum, and fever, and an increase in appetite with resulting weight gain in most patients treated with TED. When 1 gm. of the drug was given orally, about ¼ of the drug was excreted in the urine as thiamine. It appeared that not all of the TED given orally was absorbed. This compound had a stimulatory effect on the adrenocortical glands.
—I. Tateno

Cross Resistance Between Thioamide (1314 Th) and Thiosemicarbazone (in German). K. Bartmann*. *Tuberkulosearzt*, August, 1960, 14: 525–529. (*Gr. Wannsee, Berlin, Germany)

In 68 per cent of patients who excreted tubercle bacilli resistant to Conteben® (4-acetyl-aminobenzaldehyde-thiosemicarbazone), the strains were also resistant to the thioamide 1314 Th. Only 4.5 per cent of the Conteben-susceptible strains were resistant to 1314 Th. After combined treatment with 1314 Th. and cycloserine, 7 patients had become resistant to 1314 Th, and in 6 this resistence was accompanied by a marked reduction in Conteben susceptibility. Therefore, it is evident

that the development of resistance to 1314 Th cannot be prevented by the combined use of these 2 drugs.—J. HAAPANEN

How Should a Case of Open-Negative Syndrome be Treated? (in Japanese). Y. OGUMA* *et al. Jap J Thor Surg*, November, 1960, 13: 924–933. (*Kekkaku Yobokai Institute, Kiyose, Japan)

Clinical and histologic studies were made of 506 patients with open-negative cavities and 456 patients with open-positive cavities in order to determine the method of treatment indicated for the first group. (*1*) Open healing (mostly with a cavity wall of less than 3.0 mm. in thickness) was observed in only 10 per cent of all of the cavities. The longer the duration of negative sputum culture, the higher the incidence of open healing. (*2*) Reactivation after withdrawal of chemotherapy was observed in 16.1 per cent of the patients. When the duration of negative sputum culture was less than 6 months, reactivation of disease was very frequent and its occurrence depended mainly on the character of the cavity wall. It was quite frequently observed even in patients with very thin cavity walls. (*3*) Postoperative complications were seen in 6.9 per cent of the open-negative cases, a much lower incidence than in open-positive cavities. The character of the cavity wall and the duration of negative sputum on culture had no influence on postoperative complications. It is concluded that, as reactivation after cessation of chemotherapy in the cases of open-negative syndrome was seen more frequently than were postoperative complications after pulmonary resection, pulmonary resection was indicated even in cases of open-negative syndrome in which the cavity wall was thin and the duration of negative sputum culture was long.—I. TATENO

The Medical Letter*. (*136 East 57th St., New York)

December 23, 1960, 2: 101–104: Cough remedies: opiates, non-addicting antitussives, antihistamines. Chloral hydrate preparations as potent hypnotics virtually free of side effects.

January 6, 1961, 3: 1–4: Chloramphenicol (Chloromycetin®) causing aplastic anemia. Antimicrobial prophylaxis of chronic bronchitis.

January 20, 1961, 3: 5–8: Comparison tests for conformance to *U. S. Pharmacopeia* specifications of secobarbital capsules purchased from 36 drug companies. Questionable value of Reticulose® as a broad-spectrum antiviral drug. Trimethobenzamide (Tigan®) as a broad-spectrum anti-emetic. Index to volumes 1 and 2.

PULMONARY PHYSIOLOGY

Studies on the Effects of Sympathicoamines in Asthma. M. MOSKO*, M. ARKIN, B. MILLER, and G. SNIDER. *Dis Chest*, September, 1960, 38: 264–271. (*Michael Reese Hosp., Chicago, Ill.)

Ventilatory function tests were used as an objective measurement of airway obstruction. The failure to respond to epinephrine with relief of airway obstruction is not believed due to an alteration of the pharmacologic effects of the drug, but to other causes of airway obstruction not affected by sympathicoamine action. Aerosolized sympathicoamines may have a greater vasoconstrictive effect in the superficial portions of the bronchial mucosa. Subcutaneously administered drugs may have a greater effect on vasoconstriction deep in the bronchial wall and on lysis of bronchial muscle spasm. Both routes should be used in the patient with severe or refractory asthma. Small repeated subcutaneous doses of epinephrine often have a cumulative effect and have far fewer side effects than the same total dose of drug given as a single injection. No significant differences were noted in the effects of aerosol isoproterenol as compared with epinephrine.—E. A. ROUFF

The Influence of Artificial Expiratory Stenoses and of Rebreathing on the Mean Capacity in Asthma and Emphysema. P. GRONBAEK* and A. P. SKOUBY. *Acta Med Scand*, September 21, 1960, 168: 1–3. (*Municipal Hosp., Copenhagen, Denmark)

In patients with varying degrees of asthma and emphysema, artificial expiratory resistances produced increases in the mean capacity of the lungs, dependent upon the degree of resistance and the control level of the mean capacity. A decrease was never found. Rebreathing of oxygen and air caused definite increases in the mean capacity in such patients who also reacted with an increase to a given artificial stenosis. The results are discussed, with special reference to the mechanism of expiratory stenosis in patients with asthma and emphysema, and to the postulated therapeutic effect of procedures of a similar kind as practiced in the stenosis experiments (after authors' summary).—E. DUNNER

Acute Pulmonary Edema of High Altitude. C. S. HOUSTON*. *New Engl J Med*, September 8, 1960, 263: 478–480. (*Aspen Clinic, Colorado)

A case is presented of acute pulmonary edema occurring in a healthy young athlete with a normal heart. The condition is attributed to the combined stresses of cold, exertion, and the anoxia

occurring at 12,000 feet. Several other cases suggestive of a condition that may be termed "acute pulmonary edema of high altitude" are briefly reported. The mechanism of such a condition is not known, but recent observations indicate that both acute and chronic anoxia may cause striking elevation of pulmonary artery pressure, failure of the left ventricle, and pulmonary edema. Since exercise and severe cold, together with anoxia, might have cumulative effects, this explanation appears to be the most probable. The disease, often rapidly fatal, deserves further attention, and by mountain climbers in particular.—M. J. SMALL

Potential Harmful Effects of Treating Pulmonary Encephalopathy with a Carbon Dioxide Buffering Agent. A. G. SWANSON*. *Amer J Med Sci*, October, 1960, 240: 433–437. (*U. of Washington School of Med., Seattle)

Tris(hydroxymethyl)aminomethane (Tris) is an organic buffering agent which combines with carbonic acid to form a bicarbonate complex, thereby lowering blood CO_2 tension and raising blood pH. Initial trials of this agent to treat pulmonary encephalopathy produced striking hypoventilation and increased anoxemia in 3 patients with compensated CO_2 retention. The hypoventilation was probably due to the combination of Tris with free CO_2 which resulted in decreased CO_2 tension and a lessened stimulus to breathing. The anoxemia was sufficient to be potentially harmful. Buffering agents which abruptly reduce arterial CO_2 tension must be used cautiously in patients with CO_2 retention.—W. J. STEININGER

Lung Volumes and the Mechanics of Respiration in Congenital Heart Disease. C. D. COOK*, H. M. BARRIE, P. J. HELLIESEN, and S. A. DEFOREST. *Amer J Dis Child*, October, 1960, 100: 579–580. (*Children's Med. Center, Boston, Mass.)

Lung volumes were measured in 100 patients, aged 4 to 42 years, with various types of congenital heart disease. In 36, the mechanics of respiration (lung compliance and flow resistance) were also determined. In the patients with left-to-right shunts, approximately 30 per cent had significant (>2 S.D.'s) reductions in vital capacity; the group as a whole averaged 19 per cent below predicted values. Residual volume was significantly elevated in 20 per cent, while functional residual capacity was, on the average, entirely normal. Lung compliance was measured in 17 of these patients and found to be significantly reduced for the group as a whole, averaging 76 per cent of the predicted values. In 8 of the 17 cases, the compliance was only 65 per cent or less of the expected value. The work of breathing was calculated for a number of patients and found to be considerably increased. These studies indicate that in a significant number of patients with congenital heart disease, particularly those with left-to-right shunts, the basic cardiac abnormality is complicated by significant secondary changes in the lungs.
—M. J. SMALL

Effects of Acute Hypoxia and Exercise on the Pulmonary Circulation. A. P. FISHMAN*, H. W. FRITTS, JR., and A. COURNAND. *Circulation*, August, 1960, 22: 204–215. (*College of Physicians and Surgeons, Columbia U., N. Y.)

The effects of acute hypoxia and graded exercise on pressure-flow relationships were studied in normal subjects, in patients with restricted vascular beds, and in a patient with sympathetic denervation of the lungs. Acute hypoxia frequently raises the pulmonary arterial pressure in normal man. This pressor effect cannot be attributed solely to an augmented cardiac output, nor can it be ascribed to an elevated left artrial pressure. There is also some evidence that the sympathetic ganglia are not essential for the pressor effect. The exact site and mode of action of the hypoxic stimulus remain to be uncovered. This study suggests that the pressor response to acute hypoxia involves a change in the distensibility and in the dimensions of some segment of the pulmonary vascular tree.—W. J. STEININGER

Physiological Research in Chronic Pulmonary Disease: An Evaluation of Alveolar Aeration, Oxygen and Carbon Dioxide Transfer and the Pulmonary Circulation. H. MOTLEY*. *Dis Chest*, September, 1960, 38: 250–260. (*U. of S. California School of Med., Los Angeles)

The clinical evaluation of the ability of the lungs to adequately supply oxygen to and remove carbon dioxide from the blood during rest and exercise requires measurements on the ventilatory status, the transfer of gases, and the pulmonary blood flow. Single tests of lung function on only 1 aspect are unsatisfactory. There must be a more extensive application of accurate pulmonary function testing correlated with an intensive history and physical examination as well as thorough roentgenographic examination. Spirograms should be accompanied by lung volume measurements, with arterial blood and expired air studies completing the examination. There is a great need at the present time for further studies on the pulmonary circulation in chronic pulmonary disease: the oxygen uptake for the 1-minute step-up test has been helpful in detecting changes in the pulmonary circulation without the necessity for cardiac catheterization.—E. A. ROUFF

Early Fluid Administration in the Respiratory Distress Syndrome of Premature Infants. W. A. Bauman*. *Amer J Dis Child*, October, 1960, 100: 567–568. (*Columbia U., New York)

In order to test the hypothesis that early feeding of dextrose and saline solutions to premature babies lowers the incidence of respiratory distress, 50 consecutively admitted premature babies were studied. Respiratory distress was present at the age of 6 hours in 9 of 24 babies who received the dextrose and saline solution, and in 3 of 26 who served as controls. In neither group was there significant lessening of dyspnea during the first 24 hours of life.—M. J. Small

Effects of Cold Air on Respiratory Airflow Resistance in Patients with Respiratory-Tract Disease. R. E. Wells*, Jr., J. E. C. Walker, and R. B. Hickler. *New Engl J Med*, August 11, 1960, 263: 268–273. (*Peter Bent Brigham Hosp., Boston, Mass.)

Patients with chronic respiratory disease who noted respiratory embarrassment in cold environments were studied by measurement of air-flow resistance before, during, and after inhalation of and exposure to cold air. Studies revealed a marked rise in air-flow resistance in the majority of these patients when they inhaled cold (−30°C.) air. A cold-air draft delivered across the faces and torsos of the subjects while they breathed room air did not produce significant changes in air-flow resistance, nor did this exposure to cold air appear to increase the response to cold-air inhalation. Normal subjects and patients with chronic respiratory tract disease who were asymptomatic in the cold were not affected by these stimuli. The inhalation of a nebulized bronchodilating agent appeared to have a therapeutic and protective effect in the patients affected by cold. On the basis of the studies reported here, the major difficulty experienced by patients with chronic respiratory disease when they enter the cold appears related to changes in resistance to air flow. Whether this is due to spasm of the bronchial musculature, a reactive edema, excessive mucous secretions by the respiratory membrane, or other factors, is difficult to determine.—M. J. Small

Relationship of Smoking to Respiratory Complaints and Ventilatory Function. A. Wigderson* and M. Kohan. *Canad Med Ass J*, September 10, 1960, 83: 585–588. (*New Mount Sinai Hosp., Toronto, Canada)

The relationship of smoking to respiratory complaints and ventilatory performance was studied in 113 males between the ages of 17 and 99 years. The findings suggest that smoking is related to respiratory complaints. Smokers, in addition, have reduced ventilation values, but this effect is not as marked as the relationship to respiratory symptoms. The disturbances in ventilation were not found to be progressive (after authors' summary). —E. A. Riley

MICROBIOLOGY AND IMMUNOLOGY

The Metabolism of Isoniazid and Other Hydrazides by Mycobacteria. J. Youatt*. *Aust J Exp Biol Med Sci*, June, 1960, 34: 245–250. (*U. of Melbourne, Australia)

It was found that *Mycobacterium tuberculosis* (BCG) metabolized isoniazid, a hydrazone of isoniazid (verazide), and other hydrazides. These results were determined by a count of radioactive isoniazid, or its products, retained in the BCG cells after washing. Both resistant and susceptible cells oxidized isoniazid. It is suggested that the metabolism of hydrazides is not directly related to the mode of action of isoniazid but is more analogous to a detoxication mechanism. It is further suggested that cultures resistant to isoniazid metabolize isoniazid as fast as they take it up, while susceptible strains bind isoniazid more rapidly than they can metabolize it.—R. M. Ford

Diagnosis of Tuberculosis by Infection of Animals (in Spanish). J. Abello*. *Rev Esp Tuberc*, July, 1960, 29: 319–333. (*Sanatorio Helios, Guadarrama, Spain)

Specimens from suspect patients—mostly sputum and urine—were injected intrapleurally in guinea pigs or rabbits. In 1 week a small pleural effusion developed, as revealed by roentgenography. It was aspirated and subjected to the laboratory routine for demonstrating tubercle bacilli. If no pleural effusion was found by roentgenography, or if the removed pleural fluid was negative for acid-fast bacilli, a reinfection was attempted once or twice in an interval of 5 to 6 days. Two animals were usually used; 1 was sacrificed a few days after the reinfection, and microscopy and culture were performed from the pleural fluid to determine the presence of acid-fast bacilli. The other animal was sacrificed 6 to 8 weeks later, autopsied, and examined histopathologically. The diagnostic results obtained by this method are considered good.—Z. Virágh

EXPERIMENTAL PATHOLOGY

Pulmonary Adenomatosis in South Carolina Cattle. C. L. Vickers*, W. T. Carll, B. W. Bieber, J. B. Thomas, and H. D. Valentine. *J Amer Vet Med Ass*, September, 1960, 137: 507–508. (*Clemson College, Columbia, S. Carolina)

Pulmonary adenomatosis is an acute respiratory

disease, noninfectious in nature, which often but not always follows the ingestion of damaged or moldy feed. Twenty-eight cattle of 296 were infected in South Carolina during the fall and winter of 1959–1960. Of these 28, 23 died and 5 recovered. The predominant clinical sign was acute respiratory distress. Microscopic findings were hypertrophy and hyperplasia of the pulmonary epithelium. The alveolar capillaries were distended, and the bronchioles contained a mucoid exudate composed of desquamated epithelial cells. On the average, death occurred within 1 to 3 days after the onset of respiratory distress.—L. HYDE

Production of Cancers in Mice and Rats by Chromium Compounds. W. PAYNE*. *AMA Arch Industr Health*, June, 1960, 21: 530–535. (*National Inst. of Health, Bethesda, Md.)

Evidence that chromium in one or more forms is responsible for the increased incidence of cancer of the lung in chromate workers, furnished previously by the production of cancers in rats by several chromium compounds, is further strengthened by the occurrence of cancers at the site of implantation in mice receiving two of these compounds, calcium chromate and sintered calcium chromate. No tumors were produced by sintered chromium trioxide or sintered calcium chromate when injected subcutaneously in mice in which tricaprylin was used as a vehicle.—T. H. NOEHREN

Experimental Effect of Cigarette Smoke on Human Respiratory Cilia. J. J. BALLENGER*. *New Engl J Med*, October 27, 1960, 263: 832–835. (*Evanston Hosp., Illinois)

The purpose of the experimental work reported in this article was to demonstrate a possible mechanism whereby tar and other combustion products in cigarette smoke might be deposited in the lower respiratory tract of human beings. From the trachea and bronchi of human beings (or animals) a cup 2 to 3 mm. wide was used to gently "scrape" off the superficial layers of respiratory epithelial cells. Children 3 to 10 years of age, under general (vinyl ether) anesthesia, were the subjects in this experiment. The specimens were transferred immediately to a basic salt solution and stored there until prepared for microscopic observation. Perfusion with the basic salt solution alone caused no appreciable change in the rotation of the aggregates of ciliated cells. Perfusion with basic salt solution, through which the smoke of 2 cigarettes had been bubbled, stopped rotation within 5 days to 28 minutes in 12 consecutive experiments. In some cases, if the exposure to the "smoked" basic salt solution was terminated before the toxic effect was marked, return to the baseline speed of rotation could be produced by reperfusion with the salt solution alone. When room air was bubbled through the basic salt solution exactly as the cigarette smoke had been handled, perfusion with this solution had no appreciable effect on the cilia. The present experiments demonstrate how the human ciliary mechanism fails *in vitro* when exposed to smoke in solution from even so few as 2 cigarettes. It is suggested that this is one of the mechanisms whereby tars may collect in the lower respiratory tract and come into contact with the mucosa. It seems likely that the decreased efficiency of the ciliary mechanism caused by smoke plays a part in the productive cough noted by smokers.
—M. J. SMALL

Experimental Pulmonary Edema. K. BORGSTROM*, U. ISING, E. LINDER, AND A. LUNDERQUIST. *Acta Radiol (Stockh)*, August, 1960, 54: 97–119. (*U. of Lund, Sweden)

The diagnosis of pulmonary edema in patients with uremia is of significant importance. Roentgenographic examination of the lungs shows central lung changes which are marked in the hilar region, around the tracheal bifurcation, and in the corresponding parts of the esophagus. Initially, this is best seen in the lateral film. As a rule, the clinical symptoms and signs of noncomplicated uremic pulmonary edema are few. Several theories on the etiologic factors are discussed. Left heart failure and a toxic agent producing damage to capillaries are mentioned. There appears to be a striking similarity between the area of lung involved and the area of lung supplied by the bronchial arteries. In the methods and materials used, the discussion concerns the use of 16 mongrel dogs. By increasing permeability of the bronchial vessels, it has been possible to produce changes in the lung (bronchial capillary edema) similar to those seen in uremic pulmonary edema. These changes are significantly different and in contrast to the findings seen in pulmonary capillary edema and in the lung of left heart failure. Reduction in osmotic pressure of the blood produced by overhydration and/or complicating left heart failure can act as enhancing factors.—E. SHABART

An Electron Microscope Investigation of the Connective Tissue in the Alveolar Wall of the Normal and the Bronchiectatic Human Lung. E. A. LAITINEN*. *Acta Path Microbiol Scand*, Fasc. 2, 1960, 49: 136–150. (*U. of Helsinki, Finland)

An attempt was made by light and electron microscopy to compare the interstitial connective tissue in the normal and in the bronchiectatic lung. Four normal specimens were removed from the peripheral part of the lung by thoracotomy.

Eight specimens were obtained from corresponding areas in patients operated on for bronchiectasis. To avoid the local connective-tissue changes appearing in bronchiectasis, the samples were selected from areas in the lung which appeared normal macroscopically. No clear differences were found in the fibril thicknesses nor in the quantitative proportion of the collagen and the elastic connective tissue in the normal and bronchiectatic lungs. Fragmentary elastic fibers pointing to destructive changes were found in the alveolar walls of the bronchiectatic lungs by light microscopy.—E. DUNNER

Mechanisms Preventing Gastro-Esophageal Reflux in the Dog. F. G. SMIDDY* AND M. ATKINSON. *Brit J Surg*, May, 1960, 67: 680–686. (*U. of Leeds, England)

The contribution of the cardiac sphincter, the diaphragm, and the oblique fibers of the gastric wall to the competence of the cardia of the dog has been investigated. Neither cardiomyotomy nor section of the diaphragm had any demonstrable effect on the cardia's resistance to reflux. Section of the oblique gastric fibers, however, led to loss of the acute angle of entry of the esophagus to the stomach and resulted in reflux at a much lower level of intragastric pressure. It is concluded that the acute angulation of the esophagogastric junction in the dog forms the major barrier to gastroesophageal reflux.—R. MACQUIGG

Stereoscopic Study of the Inflated Lung. W. HENTEL and A. LONGFIELD*. *Dis Chest*, October, 1960, 38: 357–361. (*V. A. Hosp., Albuquerque, New Mexico)

A technique is described for the stereomicroscopic study of the fume-inflated lung. Lungs or lobes may be prepared either in the fresh state or after preservation in the deep freeze for up to 5 months. The stereoscopic examinations provide a three-dimensional aspect which is more comprehensive than conventional microscopy.
—E. A. ROUFF

PUBLIC HEALTH AND EPIDEMIOLOGY

The Incidence of Silicosis and Specific Immunologic Reactions in Lignite Miners (in Italian). E. MARINO*. *Riv Pat Clin Tuberc*, No. 5, 1959, 32: 549–558. (*Ist. Tisiol., U. of Siena, Italy)

An investigation carried out on 400 lignite miners who had been working in the mines for 8–25 years demonstrated the presence of 8 cases of silicosis and 3 of silicotuberculosis. The intradermal tuberculin reaction was positive in all 3 cases of silicotuberculosis, and in most of the cases of pure silicosis. The electrophoretic pattern showed the presence of hyperglobulinemia with a detectable increase of beta and gamma globulins.—G. CASTELNUOVO

An Analysis of Some Recent Work on Tuberculosis in Africa. C. WILCOCKS*. *Brit J Dis Chest*, January, 1960, 54: 31–39. (*Bureau of Hygiene and Tropical Diseases, London, England)

A brief account is given of the clinical types of African tuberculosis; reports on treatment generally show a favorable reaction which has considerably altered the outlook for individual patients. Although Africans tend to show the acute and severe forms of tuberculosis, there is a lot of support for the idea that this is not so much a result of inherent susceptibility as of unfavorable environmental features. These can be changed, and it is argued that the soundest measures of control are those which are equally valid for other crowd diseases, and which depend largely on administrative action and economic and educational advancement. BCG is being used increasingly in Africa and there is some evidence that there, as elsewhere, it confers partial protection. The opinion is expressed that, although it should not be withheld, it is by no means the solution of the problem (after author's summary).
—M. J. SMALL

Tuberculosis Today: Some of the World Problems and Present Trends in Management. A. SALIBA*. *Brit J Dis Chest*, January, 1960, 54: 22–30. (*District Two State Tuberculosis Hosp., Louisville, Ky.)

Probably the only reports on record showing approximately a 100 per cent reversal of infectiousness are those resulting from a daily streptomycin–high isoniazid combination. Other methods of initial therapy in tuberculosis have consisted of various combinations of streptomycin, isoniazid, PAS, viomycin, cycloserine, and pyrazinamide—all used in conventional doses. Results vary, but all investigators report therapeutic failures ranging from 3 to 10 per cent or more. When this is projected over the number of admissions throughout all of the hospitals in a particular country, the figure becomes high indeed; so that even a failure of 1 or 2 per cent need not be accepted, if these can otherwise be avoided. We are now faced with a percentage of drug-resistant tuberculous infections and, unless a more effective regimen in initial treatment is used more universally, therapeutic failures are bound to continue to increase. Thus there is a definite

need for other good antituberculous agents in the class of streptomycin and isoniazid.

Great strides have been taken in the treatment of tuberculosis, and mortality figures have been greatly reduced. Nevertheless, considered on a world-wide basis, tuberculosis remains a formidable problem. Less fortunate countries have hardly started on the road to progress. Adequate control in most western countries alone is only a partial solution to this problem. Air travel has greatly reduced distances, and more people each year use this mode of transport, so that opportunity for infection persists. Adequate chemotherapy in hospital, with or without surgery, would ensure a much better use of a limited number of beds. Once in a noninfectious state, most patients could then be managed on a domiciliary basis, thus considerably increasing patient turnover. It is highly desirable to maintain adequate chemotherapy even in home treatment and, under prevailing circumstances, the second-best choice would be the use of daily isoniazid with PAS.

Compulsory yearly roentgenographic examination for the adult population (as in the case of Australia) and compulsory hospitalization of the recalcitrant patient are steps in the right direction. The question of BCG vaccination in certain groups needs to be established. In the writer's opinion, an ideal set-up for tuberculosis treatment is that seen in England: a country-wide distribution of chest clinics with corresponding chest units either in a sanatorium or, as is the present trend, in a general hospital, provides an excellent and totally free service with regard to case-finding, hospitalization, and treatment, as well as subsequent outpatient follow-up care.—M. J. Small

Pneumoconiosis in Makers of Artificial Grinding Wheels, Including a Case of Caplan's Syndrome. E. Posner*. *Brit J Industr Med*, February, 1960, 17: 109–113. (*Stoke-on-Trent Mass Radiography Center, England)

Mass miniature roentgenography surveys in a factory producing artificial grinding wheels detected cases of pneumoconiosis, mostly of the silicotic type. All of the cases were traced to the department where the so-called "bond" is prepared and mixed with the abrasive grains of carborundum and artificial corundum. This ceramic-vitrified bond, similar in composition to English general earthenware, contained until recently a significant proportion of free silica. The miniature film survey was followed up by an investigation on full-size films, in which 92 per cent of all workers in the bond department participated. The roentgenograms were subjected to dual independent viewing, and it was found that 66 per cent of the men who had worked in the bond department for more than 10 years showed roentgenographic evidence of pneumoconiosis with a high proportion of progressive massive fibrosis. Recently, the amount of free silica in the ceramic bond has been reduced by the introduction of "frits" in place of powdered flint, and part of the factory has been rebuilt and new methods of dust suppression and dust extraction have been introduced. One of the cases described presented with the rheumatoid-pneumoconiotic syndrome first described by Caplan. It is suggested that some of the cases of pneumoconiosis, attributed to carborundum, may be due to the binding materials of artificial grinding wheels (after author's summary).—H. J. Simon

An Epidemic of Coccidioidomycosis Probably Related to Soil. B. Joffe*. *New Engl J Med*, April 7, 1960, 262: 720–721. (*Mount Zion Hosp., San Francisco, Calif.)

This paper reports the failure of a field trial to culture *Coccidioides immitis* from the air-borne fungus population at Edwards Air Force Base; an outbreak of coccidioidomycosis followed that began after large tracts of land were scraped by bulldozers and which ceased when the scraping stopped. Construction began in mid-August, and in mid-September the first of 33 adults with coccidioidomycosis was admitted to the hospital at the Base. Earth scraping stopped in December, and the last patient of this outbreak entered the hospital in February, 1959. Five female patients with serologically proved active coccidioidomycosis presented with no symptoms except skin rash. Indeed, 3 of these women never had other symptoms, and their roentgenograms showed no pulmonary infiltrates during the period of rash and on 3-month follow-up study. Previous reports of coccidioidomycosis manifested solely by skin rashes have not been made. Viable *C. immitis* has been found to a depth of 15 to 20 cm. the year around in desert soils, although viable spores have not been found on the surface during hot months. This could explain the absence of outbreaks in previous summers at Edwards Base, the failure to culture *C. immitis* from the air, and the number of cases that followed soil excavation. After soil excavation in areas endemic for coccidioidomycosis, programs for grassing, oiling, and wetting the ground should be carried out.—M. J. Small

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Prothrombin determinations require accurately prepared interstandardized reagents: Bacto-Thromboplastin, Bacto-Prothrombin 2 Stage Reagent, Bacto-Prothrombin Free Beef Plasma, Bacto-Prothrombin Free Rabbit Plasma, Bacto-AC Globulin, Bacto-Sodium Chloride 0.85%, Bacto-Sodium Oxalate Solution 0.1 Molar, Bacto-Calcium Chloride Solution 0.01 Molar.

LIVER DISEASE

Differential diagnosis of hepatic disturbances is aided by standardized reagents: Bacto-Cephalin Cholesterol Antigen, Bacto-Thymol Turbidity Reagent, Bacto-Kingsbury Turbidity Standards, Bacto-Thromboplastin and other Prothrombin Reagents.

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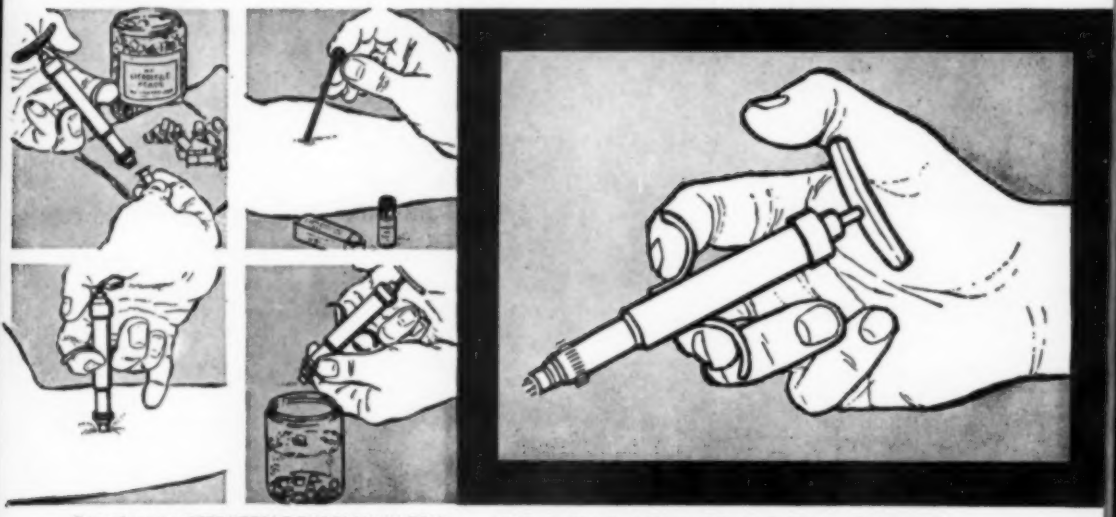

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